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CELL AND NUCLEAR DIVISION IN FULIGO VARIANS.

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(WITH PLATE XIV)

OUR knowledge of such important vital phenomena of the Myxomycetes, as the formation of the spores and the capillitium, and nuclear and cell division, is still based quite largely on the data given in the single paper published by Strasburger (23) in 1884.

Strasburger studied *Trichia fallax*, and his main conclusions were that the nuclei divide karyokinetically just before spore formation, the capillitium is formed in vacuoles, and the spores are formed by simultaneous breaking up of the multinucleated mass along hyaline lines into uninucleated spores. Strasburger found his material on decaying stumps, and speaks of finding all stages in the development of the sporanges simultaneously. Lister mentions also that the sporangia require several days to mature after their first appearance.

Strasburger describes the capillitium, which in *Trichia fallax* consists of long spirally-thickened threads tapering at each end, as arising in vacuoles of the protoplasm. These vacuoles become elongated, and the hollow tubular capillitium thread is formed in their interior. Strasburger describes the wall of the thread as being formed by the fusion of microsomes, which become aggregated in the membrane of the vacuole, and unite to form a thin, transparent pellicle. Further deposition of

microsomes in spiral lines about the so formed thread leads to the formation of the spiral ridge-like thickenings of the mature capillitium. The whole process is identical with that which takes place in the formation of a cell wall, according to Strasburger's earlier accounts of that process.

Whether or not the microsomes are the units in cell wall formation, we have here excellent evidence that the interior protoplasmic surface, which lies next to the vacuole, is equivalent to the exterior surface which forms the peridium of the entire sporange. Each surface is able in essentially similar fashion to deposit on occasion a resistent membrane over its whole extent. The doctrine of the equivalence of plasma-membrane and vacuolar membrane as developed by Pfeffer and De Vries finds strong support in this method of capillitium formation. Strasburger describes a period of nuclear division as preceding spore formation. The division is karyokinetic and the equatorial plate, separation of the daughter chromosomes, and development of the daughter nuclei are figured. The spindle fibers are inclined only slightly toward each other at the poles. The nuclei all divide at the same time, so that each section shows thousands of karyokinetic figures. Strasburger says little as to the method of spore formation. He figures the cleavage as producing the one-nucleated spores directly, and describes the boundaries of the spores as at first consisting of granules and then of clear lines, and notes also that the differentiation proceeds for the most part from the periphery toward the center. The young spore is at first polygonal, then rounds itself up and becomes enclosed by a wall.

Zopf (**27**) adheres to the view that the capillitium represents plasma masses (Hyaloplasma, Gerustplasma) which are not used for spore formation and have become hardened. He does not pretend, however, to have verified this statement for himself. Later (p. 63) he accepts Strasburger's account of the formation of the capillitium in Trichia, and is inclined to regard it as true for all the forms with hollow capillitia (Coelonemata), while still holding that the forms having a capillitium composed

of solid threads (Stereonemata) form the latter from strands of protoplasm. As a matter of fact, both forms of capillitium may arise in vacuoles, as I have been able to determine in the cases of Stemonitis and Lycogala. The account given by Strasburger is correct, and I have not thought it necessary to give further figures at this time. Whether the thread is hollow or solid, simple or branched, free or connected with the peridium or a columella, are entirely secondary conditions, depending on the extent and form of the vacuoles.

Massee neglects Strasburger's account of capillitium formation altogether, and advances the extremely loose and erroneous view that generally a surplus portion of the protoplasm takes the form of a more or less complicated network mixed with the spores, and homologous with the strands described as being present in the sporangium of Mucor, inasmuch as both structures are made from a substance separated from the protoplasm during spore formation. I have shown (4) that the so-called intersporal protoplasm of the Zygomycetes is merely excreted slime, and Strasburger's account of capillitium formation is true for all slime molds that have as yet been carefully investigated.

Lister has discovered karyokinetic division of the nuclei in the sporanges of a considerable number of genera, and has observed karyokinetic figures in the dividing swarm spores and in the growing plasmodia, though he also figures direct division as occurring at this latter stage. He concludes that whenever cell formation occurs in the life history of the Mycetozoa, the nuclei divide by karyokinesis. Lister also observed in certain genera lobed plasma-masses containing six to ten nuclei in the equatorial plate stage, and describes these masses as separating into uninucleated spores during the succeeding stages of nuclear division. For other genera he confirms the account of Strasburger, according to which nuclear division is complete before spore formation begins.

De Bary (1a) follows Strasburger in describing the spore formation for the whole group of slime molds as taking place by simultaneous breaking up of the protoplasm of the sporange

into uninucleated masses, and this is the current statement of botanical text-books.

Rosen (17) made a very thorough study, as he describes it, of *Fuligo septica*. He finds nuclei of two kinds, one poor in content and containing a so-called middle-body, and the other so densely filled with stainable substances as to appear almost homogeneous. The relative number of these two types of nuclei vary at different stages in the development of the slime mold. Nuclear division occurs prior to spore formation, but the process is described as much simpler than in the higher plants. Rosen thinks it belongs to the karyokinetic type, but it is doubtful whether a spindle figure is formed, etc. The cleavage is positively stated to be simultaneous, and to take place by the deposition of a network of granular plates which cut the protoplasm up at once into polyedric uninucleated spores. These plates are said to show microsomes very plainly, the latter being placed at right angles to the plane of the plate.

As will be seen below, my own observations on Fuligo have led to entirely different results from those of Rosen. I am convinced that his two forms of nuclei are due to inequalities in fixation such as sometimes occur. As to the method of nuclear division and spore formation I am certain that Rosen failed to find material in the stages when these processes occur. His description of nuclear division must have been based on resting nuclei whose contents happened to be somewhat unusually placed. As for the network of granular plates with microsomes such as he figures, I am convinced that no such structures are to be found in Fuligo at any stage of development. The difficulties in the way of obtaining accurate results in the study of fungus cells and nuclei are great, but not sufficient to justify such slipshod results as those of Rosen in the paper under consideration.

A summary of our present knowledge of the Myxomycetes has recently been published by Jahn (9), and reference may be made to it for a further account of the literature of the group.

My material was fixed in Flemming's solution, weaker formula, sectioned, and stained with Flemming's safranin, gentian-violet and orange.

The formation of the aethalium of Fuligo has been very well described by De Bary so far as its grosser structure is concerned. The plasmodium which is ready to form spores creeps to the surface of the substratum, and there forms a reticulum which is similar to that of the vegetative condition except that it is more dense. It becomes a rounded cake-like mass, the meshes of the reticulum being relatively small and the protoplasmic strands very thick. We have in fact at this stage a contracted reticulum, the interprotoplasmic spaces having become minute. At this stage the solids which have been imbedded in the protoplasm are all thrown out upon its surface. Large amounts of water containing salts in solution are excreted, and, the water evaporating, the salts are deposited as crystals along with the rejected solids. These waste materials are found in all the meshes of the protoplasmic reticulum, and form a sort of fragile framework piercing the ripe aethalium in all directions. The yellow coloring matter of the plasmodium is also transferred to these waste materials, so that the protoplasm is left apparently homogeneous and colorless.

A further step in forming the aethalium consists in the continued contraction of the protoplasmic reticulum, so that its superficial strands are withdrawn toward the center. In this withdrawal of the protoplasm from the peripheral parts of the mass the excreted wastes are left behind, and form thus a porous friable crust over the surface of the protoplasmic mass. At the margins of the aethalium this waste material frequently takes the form of a thin membranous almost papery border. In this further contraction of the protoplasmic reticulum the interprotoplasmic spaces become reduced in many cases to mere strands or plates of the yellow colored waste materials described above. In other cases the spaces remain as oval or angular lacunae lined with a thin yellow crust of the same excreta. *Fig. 1* shows a section through a portion of an aethalium relatively free from

such cavities. In *fig. 2*, from another portion of the same aethalium, parts of several lacunae are shown, and their relative size and distribution is thus partially indicated.

In addition to the solid particles and solutions thrown out, the protoplasm excretes over its whole surface a thin fragile membrane, in which the crystals of lime are frequently partly imbedded. This membrane is by no means as thick as in the case of the slime molds which produce sporanges, but it appears very clearly in microtome sections. In most regions it is hardly more than a cement to hold together the lime crystals in a continuous film. In other regions, where these are less abundant, it appears as a very thin homogeneous membrane. It lines all the interprotoplasmic cavities mentioned above, as well as covering the peripheral portions of the protoplasm. It is always next to the protoplasm itself. I have never found crystals or other solid excreta between it and the plasma membrane.

At a stage when cleavage is just beginning, such as that shown in *fig. 1*, the nuclei are generally in the resting condition, and are distributed rather unevenly through the cytoplasmic mass. Frequently they appear aggregated in rather dense groups in certain regions, while in adjacent regions of the cytoplasm they are less numerous. Spore development now begins with the formation of cleavage furrows, which usually arise first on the external surface of the entire aethalium and cut down at all angles into the homogeneous protoplasm. These furrows are very narrow and sharp in some cases, and quite widely opened in others (*fig. 1*). This latter condition may be due, at least partly, to a slight shrinkage in fixation. Very commonly they are curved and forked so as to cut off a superficial layer of segments. Almost simultaneously with the formation of these furrows on the surface of the entire aethalium, similar furrows are formed on the surfaces of the lacunae of the contracted protoplasmic reticulum as described above. These surfaces, of course, are in reality external surfaces of the protoplasm, and the formation of cleavage furrows from them is not in any sense to

be compared to the cleavage from vacuolar surfaces as I have described it in Pilobolus (**4**).

As noted above, the first cleavage furrows commonly do not cut through the entire mass of protoplasm in which they are formed, but curve and fork so as to cut off one or more superficial layers of segments. Further furrows, not continuous with the first, then cut through the central mass, dividing it up into large blocks, each with many nuclei. Meanwhile the superficial segments have still further divided, so that we regularly have one or few nucleated masses at the surface, while the central protoplasm is relatively undivided. The segmentation is very plainly a progressive process proceeding from the periphery toward the center. There is no such thing as a simultaneous breaking up of the protoplasm into uninucleated fragments. The protoplasm which thus segments is quite homogeneous, as noted above. There is no differentiation of hyaline zones or other specialized regions prior to the formation of the cleavage furrows. Furthermore, the nuclei show no special distribution about the cleavage planes. As seen in *figs. 1* and *2*, it is quite common to find a group of nuclei on one side of a cleavage furrow while they are lacking over a considerable area on the opposite side. There is no indication whatever at this stage that the nuclei exert any direct influence on the orientation of the cleavage planes.

If we examine the protoplasm immediately in front of one of these cleavage furrows also, we find it without differentiation of any sort which would indicate the direction which the furrow will take. Aside from the fact that it is common for the cleavage to advance along the same plane or curve in which it has started, it is quite impossible to predict in the case of any unfinished furrow, *i. e.*, one which has not yet cut through the protoplasm in which it lies, what direction it will take. It is very noticeable that these cleavage furrows do not necessarily cut the mass which is segmenting through its shortest axis, any more than through its middle. It is very common to see a strip or sheet cut off from the side of a larger mass in such a fashion that the plane of cleavage lies in the long axis of the mass which is

divided. I have even found some evidence that sausage-shaped masses are cut out of the center of larger masses by means of a cylindrical cleavage furrow. It is very common to find semi-cylindrical masses cut from the surface of the protoplasm by two furrows which curve toward each other so as to form a trough-shaped cleavage surface. All of the above varieties as to form and direction of the cleavage furrows are illustrated in *figs. 1–4*.

At and immediately prior to the time when cleavage commences in Fuligo, its nuclei are all in the resting condition. None of the nuclei indicated in *fig. 1* were dividing. A very little later, almost simultaneously with the formation of the first superficial cleavage segments, the nuclei throughout the entire aethalium begin to divide karyokinetically. In some cases it may be that the peripheral nuclei commence to divide earlier than those which lie deeper. But the difference, if it exists, generally is a very slight one. On the other hand, the process of division seems to begin progressively rather than simultaneously in different parts of the aethalium, regardless of depth from the surface. This is shown by the fact that in examining sections different stages of karyokinesis are found in different parts of the same section. For example, all the nuclei in a certain region a few hundredths of a millimeter in diameter may be in the equatorial plate stage. Moving from this region in one direction one will find a gradual transition to the anaphase stages. Moving in another direction one may find prophases, or one may find nuclei in anaphase on all sides of a region showing only equatorial plates. There is no constancy in the order of stages which will be found in moving from the peripheral to the central or deeper portions of a section cut radially to the surface of the oval, cake-shaped aethalium. It is an absolute rule, however, that widely separated stages in division are never found in close proximity to each other, at least in continuous masses of protoplasm; and generally, passing over one of the numerous lacunae, which, as noted above, pierce the aethalium in all directions, does not involve any sudden transition in the

stage of nuclear division. We may assume that the nuclei begin to divide at numerous isolated points in the aethalium, and that the nuclei of adjacent regions begin their division progressively in all or only in certain directions. If we consider that the division begins in response to a stimulus either external or internal, we should imagine the stimulus being propagated in one or several directions, from the point of its first effectiveness. Strasburger has remarked upon this same wave-like progress of the tendency to nuclear division in Trichia, and has compared the phenomena there with those in the division of the nuclei in the young endosperm of Fritillaria, in which the nuclei at one end of the embryo sac begin to divide first, and the process is then taken up progressively by the successive nuclei through the whole length of the endosperm layer.

Nuclear division proceeds thus during the whole process of cleavage, but without any relation whatever to the latter process. Karyokinetic figures can be found oriented in all possible ways to the cleavage furrows described above, and any stage in division can be found in segments of the dividing cytoplasm of any shape or size, as will be seen from *figs. 4–9*. As a further example, segments can be found with a single nucleus in any stage of division (*figs. 11, 13, 15, 16*). The details of these nuclear divisions, so far as I have been able to work them out, will be described below.

The time relations of nuclear and cell division in Fuligo are thus seen to be entirely different from those in Trichia as described by Strasburger. In Trichia nuclear division is complete before cell division begins, while in Fuligo the two processes are carried on simultaneously. The difference may be associated with a necessity for more rapid ripening of the fruit body of Fuligo. According to my observations, the building of the aethalium and formation of the spores takes place within twenty-four hours in the case of Fuligo. Strasburger does not state just how long a time is required for the development of the sporange of Trichia, but Lister found that they require from two to four days to ripen after their first appearance.

We may return now to the further consideration of the cleavage processes by which the aethalium is cut up into spores. We have noted above that the primary cleavage furrows cut into the surface of the protoplasmic mass at varying angles, that they may be curved, may branch, etc., in the most irregular fashion, with no reference whatever to the distribution of the nuclei. This much of regularity, however, can be seen. The furrows are so oriented with reference to each other and to the surface of the mass that cleavage at first progresses more rapidly at the surface than in the center. Thus, one or more layers of very irregular one- to several-nucleated segments are cut off on the periphery, while the central mass has been cut through by only a few furrows. What is true of the exterior of the aethalium as a whole is also true of the surfaces of the interprotoplasmic gaps or lacunae. It can be seen from *fig. 2* that the surface of each such lacuna is lined by a layer of one- or few-nucleated segments, while beneath them larger multinucleated segments are found. The peripheral segments are very irregular in shape, as are also the larger central segments. Frequently broad thin plates are found; elongated sausage-shaped masses are also common. As noted above, the cleavage planes follow no such simple rules as cutting through the short axis of the mass to be divided, or always dividing a mass successively in planes that intersect at right angles. Hofmeister's law, also, that cell division always occurs transversely to the axis of most vigorous growth, has no application here, since no growth is taking place at the time when these divisions occur.

If we study the cleavage of any one of these central masses of protoplasm we shall find the orientation of the furrows essentially similar to that of those which cut in from the surface of the entire mass. *Fig. 5* shows such a mass with its nuclei all in the equatorial plate stage, both polar and profile views of the latter being shown. It is to be especially noted also that the furrows in *fig. 5* are not directly continuous with those that appeared first on the surface of the mass from which the segment in question was taken. No single furrow can be traced

for any great distance in an unbroken plane or curve. The furrows which are to further subdivide the segment represented in *fig. 5* have been formed independently of those by which the block itself was delimited. The plan seems to be that each furrow may cut through the mass in which it originates, but may not continue across the furrows with which it intersects so as to cut through successive masses in any specific direction. There are no general planes of cleavage for the whole mass. As noted already, the first furrows that form do not as a rule cut deep down into the mass toward a center. Rather they branch or are curved so as to cut off irregular blocks on the surface. New furrows forming on the surface of these, and at very varying angles with them, continue the cleavage into the deeper portions of the mass.

It is well shown in *fig. 5* that the furrows at this stage also cut into a perfectly undifferentiated mass of protoplasm, there being absolutely nothing by which to predict the path they will take except the general direction which they have already entered upon. The protoplasm is singularly homogeneous, without large vacuoles or inclusions of any sort, and through this undifferentiated mass these furrows are formed. It is further seen that they may be either plane or curved, and lie at various angles with each other and the surface of the mass.

This type of cleavage results in no very definite conditions as to the size of the segments formed. Still we can find a stage when the peripheral segments of the mass are quite regularly uninucleated and the deeper portions have been cut to various dimensions, each containing from eight to sixteen nuclei. So far the process resembles that already described for Synchitrium; but at this stage a very noticeable difference in the method of division makes its appearance. This difference is shown in *figs. 6–9*. Whereas, hitherto, there has been absolutely no differentiation of the protoplasm to mark the path to be taken by the cleavage furrows, now broad hyaline areas are formed midway between each pair of dividing nuclei. These are not at all hyaline zones of equal thickness, but furrows broader at the surface

and narrowing toward the centers between each pair of nuclei. The surface is generally slightly depressed in the line of these hyaline regions, indicating the beginning of the furrow which is actually to sever the portions thus preliminarily marked off by the hyaline regions.

The appearance is as if all the denser portions of the protoplasmic mass had contracted about each nucleus as a center, thus leaving irregular, furrow-shaped, less dense spaces in the middle region between each pair of nuclei. These areas contain very little or no stainable material, and seem to be filled with a watery liquid merely. They are, however, not in any sense rounded vacuoles whose cell sap shows surface tension where it comes in contact with the denser protoplasm. The surface of the rounded mass of protoplasm aggregated about each nucleus is by no means smooth and even, as is the surface of the protoplasm about a vacuole. The denser protoplasm passes over by insensible gradations into the less dense material in a fashion very hard to reproduce in a drawing. Peripherally these hyaline areas are bounded by a very thin protoplasmic film consisting of little more than the plasma membrane itself, which can here be more perfectly recognized as a distinct membranous film than in any other condition of cell development which I have yet observed. The plasma membrane is in these stages never broken through. It always forms a perfectly continuous enveloping layer surrounding the entire segment which is being divided, as is shown in *figs. 6–9*. In addition to the existence of these hyaline areas which predetermine the future cleavage planes, another striking condition is to be noticed. The hyaline regions in Fuligo bound off in every case a single nucleus and never a group of nuclei. This nucleus may be in process of dividing, but the daughter nuclei are never completely reconstructed at a stage when these hyaline regions are present cutting off the pair. The segregation is about the nuclei as units, and the cleavage thus predetermined is to be a cleavage by which the entire mass will be cut into uninucleated segments. Whereas hitherto the cleavage planes in these central masses have been

oriented without especial reference to the distribution of the nuclei, thus cutting off larger and smaller segments with more or fewer nuclei, they are now to proceed midway between each pair of nuclei, so that equal and uninucleated masses will result. As already noted, the impression is very strongly given by these dense rounded masses separated by relatively watery regions that a contraction has taken place about each nucleus as a center. Such conditions might be produced by a contraction originating in the structure of the cytoplasm itself, or in a pull exerted upon the cytoplasm by the nucleus. After the formation of these hyaline areas, the cleavage is completed by the furrowing of the plasma membrane along the lines marked out. A later stage than that shown in *fig. 6*, in which certain furrows have already cut deeply down into the hyaline regions, is shown in *fig. 7*. The furrows are apparently formed just as they were in the earlier stages, but they follow the hyaline areas, and thus the separation of the uninucleated masses is completed. Such cleavage as this is progressive in the sense that both the hyaline regions and the constriction furrows are developed gradually from the surface inward. When complete, however, it results in the simultaneous production of a number of uninucleated cells equal to the number of nuclei in the original mass. And in this respect it differs markedly from the cleavage in the earlier stages, in which larger multinucleated masses are progressively cut up into segments with fewer and fewer nuclei. I have already figured a similar differentiation of a hyaline region predetermining the plane of cleavage in the formation of the spores of Pilobolus (**4**, *pl. 25, fig. 21*). In this figure resting nuclei are shown in two groups, between which a less dense zone is formed, through which later, as is shown in *fig. 22*, a cleavage furrow passes. The appearance was little regarded in my description of cleavage in Pilobolus, but its appearance at a similar late stage in the cleavage of Fuligo indicates that it may have considerable significance in connection with the relations of the nuclei to the cleavage phenomena. The hyaline areas, in Fuligo at least, appear at about the stage in cleavage when the furrows

seem to be more definitely oriented with reference to the distribution of the nuclei. At the stage of embryonic growth, when they are found in Pilobolus also, cell division and nuclear division are proceeding in a somewhat definitely correlated fashion. It can hardly be questioned that whereas in earlier stages the cleavage was largely independent of the nuclei, it comes later to be directed solely with reference to their distribution, and it seems not unnatural to assume that in this latter stage the nuclei control the orientation of the cleavage planes. If this is the case, it is quite possible that the formation of the hyaline zones is the visible expression of this activity of the nuclei.

On the other hand, it is quite possible to assume that cleavage throughout is controlled by the cytoplasm, at first with little reference to the distribution of the nuclei, but later with special reference to the formation of uninucleated cells. The formation of hyaline zones preceding the cleavage furrows might in this case also mark the transition from the earlier irregular to the later more definitive stage of cleavage without implying any special activity of the nuclei. I have already noted that it is quite as easy to assume that the cytoplasm itself contracts about the nuclei as that it is drawn together by a tension exerted from the nuclei. Either view is consistent with the assumption that material for the growth of the plasma membranes is formed in the nucleus and passes outward from it to the newly-forming cell boundaries. As is seen from *figs. 6–9*, the nuclei are dividing while the cleavage just described is going on, so that the uninucleated segments formed become almost immediately binucleated. Cell division then follows either before or after the complete reconstruction of the daughter nuclei (*figs. 16, 17*). Thus, in the end, uninucleated spores are produced. The formation of a hyaline region and constriction furrow for the division of a binucleated cell whose nuclei are already in the anaphase stage is shown in *fig. 9*. The beginning of the constriction for the final division of a binucleated cell to form two uninucleated spores, the hyaline region having not yet appeared, is shown in *fig. 16*.

Transition types of cleavage between that without and that with a preliminary formation of a hyaline furrow are abundant. *Fig. 8* shows a six-nucleated mass of protoplasm dividing by furrows, which in three cases are preceded by hyaline differentiation, and in the other two cases are cutting directly into the undifferentiated protoplasm.

With the formation of uninucleated segments whose nuclei divide no more, the process of cleavage is complete. As noted above, the cleavage results in uninucleated segments at the periphery of the protoplasmic masses much earlier than in their interior. The nuclei in these early formed segments are always found dividing. The definite spore cells with a single resting nucleus are probably formed first in those regions of the aethalium where cleavage first began. The final delimitation of the spores seems to proceed progressively from these regions in all directions through the aethalium. Fully formed spores may ultimately be found throughout the greater part of the aethalium, while in certain regions here and there cleavage may still be in progress. There is, however, in the later stages of cleavage no such marked difference between peripheral and central regions as there was during the early stages. Whether this is due to retardation in the cleavage at the periphery during the later stages, or whether the nuclei there divide repeatedly to prolong the process, I have not been able to determine.

Summarizing, we may characterize the whole process as one of progressive cleavage by means of furrows which cut through the protoplasmic mass in very many directions and at very varying angles to each other. The process is progressive both in that the furrows originate on the surface and proceed gradually toward the center, and in that larger multinucleated segments are first formed which are by further divisions reduced to the condition of uninucleated spores. It may perhaps be distinguished from bipartition as a process of successive multipartition, since cleavage furrows may invade any particular portion of protoplasm simultaneously from a number of directions. At first the orientation of the cleavage planes shows no evident relation

to the distribution of the nuclei. Later the furrows proceed in every case so as to cut off uninucleated masses approximately equal in size. This later period of cleavage is characterized in many cases by the aggregation and rounding up of the denser cytoplasm about the nuclei so as to leave hyaline regions midway between each pair of nuclei, thus predetermining in each case the plane of cleavage to be followed later by the cleavage furrow. This type of cleavage results immediately in every case in the formation of uninucleated cells whose nuclei, however, may still be in a state of division. In the end, the entire protoplasm of the aethalium has been cut into uninucleated segments which are at first naked bits of protoplasm. Later each cell becomes surrounded by a wall and constitutes a spore.

Turning now to the phenomena of nuclear division, we may note first of all that the structure of the resting nucleus conforms, in spite of its small size, to that of the nuclei of other fungi and the higher plants. Nuclear membrane, chromatin (nuclein), and nucleole are present, and are differentiated by staining with safranin, gentian-violet and orange, just as sharply as they are in the pollen mother cells of the lily. The nucleole frequently lies in a clear space (*fig. 10*), as is so frequently seen in the nuclei of the root-tip of the onion.

The nuclei, however, are too minute for the successful study of the prophases in spindle formation. In the equatorial plate stage (*figs. 3, 5, 11*) the spindle is sharply differentiated. It shows rather sharp-pointed poles which may be more densely stained at their tips. Broad-poled spindles, such as those figured by Strasburger for Trichia, are not found in Fuligo. The chromosomes stain deeply in the equatorial plate stage. In polar views it is possible to count the number with considerable definiteness. The great number of these figures to be found in sections of Fuligo at this stage make the material especially favorable for such study. The chromosomes are relatively short and thick, and form a very regular equatorial plate, all of them lying practically in a single plane, so that in polar views they are practically all in focus at once. From a study of a large number of

such figures, I am quite certain that the number of chromosomes is twelve.

All stages of the separation of the daughter chromosomes and their migration to the poles of the spindle can be found in the greatest abundance (*figs. 6, 8, 12–15*). The spindle becomes slightly elongated during this process. Connecting fibers are present and form a figure closely resembling that in the corresponding stages in the lily or larch. As the chromosomes first separate, relatively large gaps are seen between the connecting fibers, which appear bunched together in a few large strands (*fig. 13*). The whole connecting spindle is markedly barrel-shaped at this stage. As the chromosomes approach the poles, the connecting fibers become more evenly distributed, and are straightened so as to form a cylindrical series extending between the groups of daughter chromosomes (*fig. 14*). A marked difference between the nuclear divisions in Fuligo and those in the asci I have studied is seen in the arrangement of the daughter chromosomes as they are drawn back to the poles. In the asci these chromosomes are widely scattered on the spindle at this stage, some having nearly reached the poles, while others are much nearer the equatorial region. This condition makes this stage the most favorable for counting the chromosomes of the nuclei in the ascus. In Fuligo, on the other hand, all the daughter chromosomes retreat simultaneously toward the poles, as is seen in *figs. 6, 13, 14*. They also become quite densely massed together, so that the individual chromosomes are not so easily distinguished in polar views at this stage as in the equatorial plate stage.

As the daughter chromosomes reach the poles, the whole figure is still more elongated, the connecting fibers being drawn out (*fig. 15*) into a long slender strand, which gradually disappears. The poles of the spindle can be distinguished beyond the groups of daughter chromosomes till a very late stage (*figs. 9, 15*).

The nucleole of the parent nucleus in Fuligo disappears at a rather early stage as compared with other fungus nuclei. It is

never to be found lying midway between the daughter nuclei near the old spindle, as is regularly the case in many asci. The daughter nuclei are apparently reconstructed in the ordinary fashion. The figures, however, are too small to show very characteristic details at this stage.

The similarity of the whole process of nuclear division in its main outlines here to what is found in higher organisms is certainly very striking, and shows clearly enough that simplicity of structure and life history on the part of the whole organism is by no means to be taken as indicating a corresponding reduction in the complexity of the nuclear structures and activities.

The capacity of the slime molds to become encysted at any stage in their life history when conditions become unfavorable is very well known. A condition which I have sometimes found, and which is represented in *fig. 19*, indicates that this may occur midway in the process of cleavage. The aethalium in question was made up of rounded, two- to several-nucleated masses, each provided with rather a thick wall. Whether later with a return of favorable conditions such masses would continue their cleavage, and form normal uninucleated spores, or whether they would themselves function as spores, I have not been able to determine. A normal uninucleated spore is shown in *fig. 18*.

The aethalium and the sporanges of the Myxomycetes differ from the sporanges of Synchitrium, Pilobolus, and Sporodinia, whose method of spore formation I have already described (**4**), in that the multinucleated condition in the former originates at least in the formation of the plasmodium. The plasmodium is a product of cell fusions without nuclear fusions, so far as known at present. Physiologically considered, in all its functions of nutrition, growth, and response to external stimuli, it is the equivalent of such multinucleated masses of protoplasm as are formed simply by growth and nuclear division without cell division. The plasmodium itself increases its original volume, as formed by fusion, by this same type of growth. Fundamentally considered, it is the physiological equivalent of the multinucleated mass formed in Synchitrium by the division of the original

single nucleus of the vegetative body. The internal relations of nuclei and cytoplasm cannot be conceived as different, merely because in one case the nuclei and separate cytoplasmic masses were brought together by fusion, while in the other they were formed from a uninucleated cell by growth and nuclear division.

Morphologically, however, the two structures must be regarded as entirely distinct, and the possession of the plasmodium, and the capillitium formed as a deposit in vacuoles by the slime molds, is probably sufficient reason for regarding them as constituting a separate developmental series running back to an origin independent of any of the existing groups of algæ or fungi. Sachs (**18**) has quite recently expressed the opinion that they are to be classed with the fungi, but he brings no morphological evidence to support his view. There can be no question that the Acrasieæ represent simpler forms out of which the Myxomycetes have developed, and we thus have a developmental series leading from simpler to more complex forms. The plasmodium and capillitium, appearing only in the more specialized members of the group, are plainly secondarily acquired structures developed as additions to the structural features of the Acrasieæ, and are not to be directly homologized with physiologically equivalent structures in other groups.

The physiological equivalence of the plasmodium and the multinucleated masses of protoplasm found in other fungi, such as Synchitrium, Pilobolus, etc., can hardly be questioned. Elsewhere (**3**) I have discussed the question as to whether these multinucleated masses should be classed as single cells, or as the equivalents of many-celled tissues or organisms. The plasmodium of a slime mold is well calculated to furnish further evidence on this point. In its method of origin by the fusion of distinct amoeboid swarm-spores, it would seem to testify to its multiple nature; still, as noted above, the whole physiology of the plasmodium in its nutrition, reactions to stimuli, and growth, shows most conclusively that it is a unit in exactly the same sense as is an amoeba, or one of the swarm-spores which combined to form it. In fusing, the swarm-spores gave up their individuality

to become parts of a larger mass. That this fusion did not involve a fusion of their nuclei cannot be considered as altering the result so far as the question of individuality is concerned. Where sexual cells fuse and their nuclei unite there is no question that the resulting fertilized egg is a single cell. If, as Häcker (2) has shown is the case in Cyclops, the pronuclei remain distinct through the early cleavage stages of the egg, this cannot be taken as evidence that the two nucleated bodies thus produced are not single cells rather than the equivalents of tissues. These binucleated cells, functionally and morphologically considered, are the equivalents of the later cells of the Cyclops which appear with a single nucleus. The conclusion must be, as I have already pointed out, that the individuality of the cell is independent of the number of nuclei which it contains.

Hertwig argues for the *potential* equivalence of multinucleated cells and tissues. The word potential here of course may mean much or little. In support of his view he urges the case of the insect egg, whose nucleus divides to form hundreds of daughter nuclei before cleavage begins. Later the multinucleated yolk mass is by cell division separated into a blastoderm of as many cells as there were nuclei present. It is quite plain, says Hertwig, that the apparently simple egg could not with a single stroke, as it were, have become a multicellular organism. The question here, of course, is how great a change is involved in the transition from the one-celled to the many-celled condition, and on this point it is interesting to note that up to the stage when cell division takes place in the insect egg there has been no visible differentiation of embryonic structures in the egg. The cel division simply transforms the one cell into a mass of equivalent cells, and this need hardly be considered as a change too great to be due entirely to the cleavage process. The relation of multinucleated and uninucleated cells is well shown in the very fact that the visible differentiation of the insect embryo, aside perhaps from the determination of its axes, which was accomplished even earlier, begins after the division of the egg into numerous cells, and not while it remains a single cell, although

it has meanwhile become multinucleated. It would seem that differentiation was dependent to a certain degree, at least, on the interaction of individualized protoplasmic units, each capable of receiving and reacting to independent stimuli as the parts of a multinucleated cell cannot. Hertwig, in his doctrine of biogenesis, himself insists on the importance of the interaction of separate cells for the production of physiological differentiation and division of labor.

To be sure, we have abundant evidence that the multinucleated cell can achieve a certain degree of differentiation, as is shown in the numerous Siphoneæ which mimic in their root-like, leaf-like, and stem-like structures, the analogous parts of the higher plants. It is perfectly apparent, however, that this differentiation is on a far simpler scale than is seen in the complex mechanical and other tissue systems and organs of the higher plants. Indeed, the relative unimportance of the Siphoneæ as a part of the earth's vegetation is to be regarded as very strong evidence that the type of structure which they show in their multinucleated cells is by no means well adapted to develop complexity and differentiation of structure such as is necessary to meet the manifold variations in environmental conditions to which all plants are subjected. These Siphoneæ are after all hardly more differentiated than the infusorians, which are typically unicellular. Pfeffer (**16**) puts the case very strongly when he points out that we can conceive of no such independent units in the multinucleated cell as the energids of Sachs are defined to be. If the energid is a nucleus with a portion of cytoplasm under its immediate control, there can be no such structures in the Siphoneæ, since the protoplasm of their cells is constantly streaming from one point to another, with the exception of the plasma membrane, which remains fixed. No nucleus could thus have any definite relations with any particular portion of the plasma membrane, and it is hard to conceive that in this streaming motion any portion of the semifluid cytoplasm should remain constantly in connection with any particular nucleus. As Pfeffer says, we must conclude that any specific mass of cytoplasm in a

multinucleated cell will be simultaneously influenced by various nuclei which are in contact with it. There can be no invisibly bounded units in which the same living substance remains united. Pfeffer also justly objects to Sachs's characterization of the Siphoneæ as *noncellular* plants, and regards them as both morphological nnd physiological units.

If we compare the method of spore formation in Fuligo with that which I have described elsewhere (4) for Synchitrium, Pilobolus, and Sporodinia, it will be seen that the processes in all these forms are identical in their main features, while differing in a number of important details. In the four cases the cleavage is progressive from the surface inward, larger segments being first formed, which are later cut up into uninucleated cells, except in *Synchitrium taraxaci* and Sporodinia, in which the multinucleated segments function directly as spores.

In the earlier stages of cleavage in Pilobolus and Fuligo the furrows pierce through perfectly undifferentiated and quite homogeneous protoplasm, while in the later stages the differentiation of hyaline areas, wedge-shaped in transverse section and cutting through the masses to be divided, predetermine the planes of the cleavage furrows. Such hyaline areas were not observed in Synchitrium or Sporodinia. In Synchitrium and Sporodinia nuclear divisions precede cleavage. In Pilobolus nuclear divisions occur during the later stages of cleavage, and in Fuligo nuclear divisions and cleavage proceed simultaneously throughout.

Fuligo is the only one of the five forms in which the uninucleated segments formed by the completion of the cleavage process, and which I have called protospores, become the functional spores directly without further growth or nuclear division. In this respect perhaps the cleavage of Fuligo represents a more simple primitive type than that of either of the others.

In all forms the orientation of the furrows with reference to the surface of the dividing mass and with reference to each other is extremely varied, and it can be laid down as a general rule for the forms studied that no one furrow can be traced

continuously through the entire sporange or aethalium which is to be divided except, perhaps, in the case of the very thin layer of spore plasm in Sporodinia. On the contrary, by the curving and branching of the furrows, segments irregular in their size, shape, and number of nuclei are cut off successively from the periphery toward the center. These segments in turn, and also progressively from periphery to center, are cut up by new furrows into smaller segments, until finally in *Synchitrium decipiens*, Pilobolus, and Fuligo the uninucleated condition is reached. No general system of cleavage planes, either parallel or radial to the surface of the dividing mass, can be discovered. The path of the cleavage planes as division progresses becomes an inextricable confusion of zigzag lines, branching and intersecting at almost every angle. The occurrence of such similar types of cleavage of the multinucleated mass as are found in the aethalium of Fuligo and the sporanges of the Phycomycetes must be regarded as another example of parallel development in structures not phylogenetically connected. The explanation of the similarity in these forms of cleavage is to be sought in the fundamental physiological properties of protoplasm, and not in hereditary transmission to the different branches of a series of genetically related forms.

With the above account of fusion in Fuligo representing the Myxomycetes, types of all the main groups of fungi producing asexual spores in the interior of mother cells have been described except the Oomycetes, and while it will be necessary to investigate representatives of all the genera, at least, in these groups, still the hypothesis is fairly justified that some form of progressive rather than simultaneous cleavage by cell plates will be found in every case. Klebs's (**15**) investigations of Hydrodictyon also indicate that the formation of zoospores is at least not by simultaneous division into uninucleated segments, and the whole process in this alga should be further investigated, especially with reference to the occurrence of the nuclear fusions which Klebs describes as occurring in the developing zoospores. It may be noted here also that Bachmann (**1**), in describing a new species of Mortierella, has observed incidentally the marking off of the

surface of the sporange into irregular polygonal areas at the time when spore formation is beginning. The lines marking off these areas are doubtless the beginnings of the cleavage furrows as I have described them for Pilobolus and Sporodinia. Bachmann made no sections, but concludes that the spore formation must be a progressive process.

A sufficient number of forms has been investigated to show that progressive cleavage is a widely spread phenomenon among the lower plants, occurring in very many cases when multinucleated masses are to be cut up into smaller cells. That simultaneous cleavage may also occur is quite possible, but the evidence for it is not strong except, perhaps, in the case of the sporange of the Saprolegniaceæ.

I have already shown elsewhere (**4**) that the progressive cleavage of the sporange in spore formation is in principle the same process as that which has been described as division by constriction in Cladophora and the conidiophores of the mildews, and it will be of interest to attempt a comparison of this progressive cleavage with cell division as found in the growing points of the higher plants, especially from the standpoint of the more general theories of cell division.

Schleiden's (**21**) doctrine that the form of a plant is determined by its cellular composition, including as the two important factors the arrangement of the new-formed cells in growing regions and the subsequent varying growth and enlargement of these cells in their three dimensions, was first opposed by Hofmeister. Hofmeister (**7** and **8**, p. 129) advanced the view that cell-formation is subordinate to the growth of an enlarging organ taken as a whole. He held that the growth of the single cells of a vegetative point is controlled and conditioned by some formative principle which determines the growth of the entire organ, this latter being directed toward simple enlargement or the development of some predetermined form. According to this view, growth of the vegetative point cannot be interpreted as determined by the sum of innate growth tendencies of the individual cells.

Hofmeister believed that cell division takes place according to a simple mechanical law. The new cell wall always cuts the axis of most intense growth at right angles (**8**, p. 127). It is plain that such a principle as this can have no application in interpreting the division of the masses of protoplasm in sporanges whose growth is complete, the accompanying tensions, as may be fairly assumed, having also reached a condition of equilibrium.

Sachs (**20** and **18**, p. 22) follows Hofmeister in regarding the growth and division of the single cells as subordinate to the growth of the vegetative point as a whole. From a study of the arrangement of the cells in the growing point of the higher cryptogams and flowering plants, he has developed the law of the rectangular intersection of cleavage planes in the successive cell divisions. He regards this as the most universal law of structure in the plant world, and holds that it is independent of all phylogenetic relations, and not a result of natural selection. As is well known, he holds that the outer form of the growing organ is the primary determining factor for the orientation of the cleavage planes. The periclines conform directly to the surface of the embryonic organ. The anticlines cut the periclines at right angles, and if division is to occur in three dimensions the transversals also appear in a third plane at right angles to the other two. Sachs believes that the relations thus expressed are of so fundamental a nature as to be comparable to those determining the relations of the axes of a crystal. The cellular structure of a young organ is related to these *Leitlinien* as the structure of a crystal to the arrangement of its faces and their intersecting angles. The form of a growing point is determined by phylogeny, and selection when given the direction of the cleavage planes, is at once known. Such fundamental relations as these, occurring in the most widely separated groups, are to be considered as innate in protoplasm. Sachs proposes to call them mechanomorphoses. The principle thus developed by Sachs has been generally accepted as explaining the arrangement of the cell walls in the growing points of the higher plants,

and, as Sachs himself notes, the investigation of the apical cel- and its divisions has ceased to be regarded as affording a key to the explanation of the development of shoots.

Assuming the correctness of Sachs's law for the higher plants, if we attempt now to apply it to the case of the cell-division in sporanges we are confronted with many difficulties.

First of all, these sporanges do not divide by successive bi-partitions as do the cells in the growing points referred to. Nor do they divide by simultaneous delimitation of the energids which, according to Sachs's view, compose them. Their cleavage is progressive, and the cleavage planes, as shown in sections, form no great series comparable to the anticlines and periclines which Sachs finds in a section of a root tip. In the cleavage of the sporange the principle of rectangular intersection is violated constantly, the angles of intersection of the cleavage planes showing no constancy whatever. It may be objected that the sporange is not a growing organ, and hence its method of division should not be expected to conform to that of growing points. Growth is complete in the sporange before division begins, though it may recommence in the later stages of cleavage in Pilobolus. The process in the sporange consists in cutting up into cells a mass of protoplasm whose form has been already determined and its growth completed. Still, although Sachs states the principle of rectangular intersection for growing points, and conceives it as determining the arrangement of the cells in the growing point as it pushes forward in the elongation of the shoot, he always conceives the divisions as occurring in these growing points after the essential embryonic growth of the cell concerned is at an end. Growth of cells subsequent to division may, and generally does, in his opinion, distort the relations of the cleavage planes.

Sachs makes no attempt to include the multinucleated sporangia in his discussion. Still he specifies the growth of the Siphoneæ (**19**, p. 100) as exceptional when compared with the irregular growth of many thallophytes, and considers the development at their growing points as typical of that in the higher

plants. He also specifically states that typical mechanomorphoses are the cell nets in growing points of young organs *and structures whose cells show no individual growth after cell division is complete*. All the sporanges mentioned above would be included in this latter type of structures. The sporange of Synchitrium is a spherical mass of protoplasm which divides into cells which show no further growth, at least till after cleavage is complete. At this stage, then, the cleavage planes should illustrate typical mechanomorphosis. It would seem that in such a spherical mass of undifferentiated protoplasm the opportunity for the law of rectangular intersection to come to full expression would be especially good. We might expect the periclinal and anticlinal cleavage planes to be extremely conspicuous in such a case. On the contrary, as noted above, and as is well shown in *figs. 2, 4, 14, 15, pl. 24*, of my former paper (**4**), no regular periclines and anticlines are to be observed. The surface furrows cut into the mass at very varying angles, frequently also becoming curved and branching so as to intersect near the surface, and thus cut off superficial cell-masses of the most irregular shapes and sizes. There is apparently the greatest irregularity in the orientation of the cleavage planes both with reference to each other and to the surface of the dividing mass, as a glance at the figures referred to above will show, and as I have described more fully in the case of Fuligo. As this method of progressive cleavage, however, is of wide occurrence among the thallophytes, the principle of rectangular intersection loses that universal validity for cell division upon which Sachs so strongly insisted. Sachs uses the law of rectangular intersection so as to further support Hofmeister's doctrine of the subordination of cell division to the growth of organisms as wholes, and in this sense his results have, for the most part, been taken up and utilized for theoretical purposes in discussions on the nature of the cell and its division (see **6** and **26**). With Hofmeister, Sachs considers that the growing point of such a structure as the pine shoot develops simply as a mass of protoplasm analogous to that at the growing end of a Vaucheria filament. The shape of the end

of the shoot has been determined by selection during phylogenetic development; its growth is the enlargement of a protoplasmic mass along lines predetermined. The putting in of the cell walls, being a purely mechanical process following the simple rule of rectangular intersection, can in no way be regarded as determining the shape of the shoot. The tip grows just as does the Vaucheria tip, the formation of individual cells being a purely secondary matter. The independence of the single cells, their growth and division, is entirely subordinated to the growth of the entire organ. It is an open question whether the rectangular intersection of cell walls in vegetative points, assuming that the facts are as Sachs describes them, is sufficient basis for so important a conclusion as that above stated.

Jennings has characterized the law of rectangular intersection as "hardly to be considered as more than a statement of a condition commonly found." There is nothing inherently impossible in the assumption that the habit of forming successive cell plates so that they intersect at right angles has itself been acquired by the cells as individual units. The form of the shoot may then depend on this power of the cells, assuming always a further regulation of their activities by reciprocal stimuli between the cells themselves as well as by stimuli from their environment.

There can be no question that at least the details of cleavage in the sporanges I have studied have been modified in the course of phylogenetic development with especial reference to the needs of the organisms concerned. I have elsewhere pointed out the correlation between the abbreviated cleavage process in Sporodinia and its more rapid spore development. The participation of vacuoles in the formation of the cleavage furrows in Pilobolus is another example of such modifications. That the cleavage processes are after all so similar in the different sporanges studied is doubtless due to the fundamental physical and chemical properties of the protoplasm, but the process is none the less to be conceived of as modified by selection. It

must also be borne in mind constantly that in such cleavage phenomena as these the cleavage planes are influenced in no way by the need of forming any specific tissue or plant form In the growing point of a metaphyte it may perhaps be difficult to decide whether the planes of division are determined by the cells themselves in accordance with internal conditions, or whether, as Sachs claims, it is the shape and differentiation of the shoot taken as a whole which determines the planes of division. In the division of these spore-forming masses no such question can arise, since no differentiated tissue is to be formed. The problem is simply to divide the large mass into smaller masses more convenient for distribution. In doing this, as I have shown, entire irregularity prevails as to the orientation of the cleavage planes with reference to each other, and with reference to the axes of the mass to be divided. It may be concluded that the protoplasm is *per se* perfectly isotropic so far as cleavage is concerned, and that it is a matter of indifference whether the cleavage planes intersect at right angles. If rectangular intersection is the rule in the higher plants, it might well be argued that this is a secondarily acquired condition, assuming with Pfeffer that these multinucleated structures are single cells.

The question can hardly be raised in this connection whether the organism or the cell forms the new cells or spores. Still it is interesting and significant to note, as I have pointed out already, that the process of cleavage in these sporanges is essentially similar in principle to the typical cell division by constriction, which I have described as producing the one-nucleated cells at the base of the conidiophore in the mildews, and which is also found in Cladophora. Progressive cleavage by surface furrows is only a more complex modification of cell division by constriction. Spore formation is simply cell division, though not by repeated bipartitions of the mother cell, as it commonly occurs in the vegetative growth of the higher plants.

The growth of the Multinucleatae cannot then be regarded as illustrating the growth of an organism comparable morphologically to a metaphyte but without cell formation. The

cœnocyte is a cell, and its growth and differentiation is comparable to the growth and differentiation of a single cell in the growing point of one of the higher plants. That such a cell may become multinucleated is illustrated by the multinucleated cells in the red algæ and the striped muscle fibers of animals Just as in these cases, the cœnocyte everywhere is a cell which has bcome multinucleated strictly for functional purposes requiring the distribution of the nuclear material through the enlarged cell body.

The more modern theories as to the division of the animal cell all assume a definite correlation between nuclear and cell division. The mechanism of the one is definitely connected with that of the other. The division of the cell at right angles to the long axis of the karyokinetic spindle is the rule among the higher animals as in the higher plants, and the later theories have assumed this relation as fundamental. Thus, Heidenhain's (**5**) theory assumes that division of the cell is a result of tensions in the unequally stretched elements of a "system of organic rays" extending from the centrosome to the plasma membrane. Kostanecki (**11**) attributes cell division to the development of a cell plate which is formed as a result of the migration of the ends of pairs of polar rays, produced by the splitting of parent rays, from the points of their original attachment to the plasma membrane of the mother cell into the equatorial region between the daughter nuclei. Jennings (**10**) has summarized the theories of cell division and of the orientation of the successive cleavage planes in animal cells, and I need not enumerate them here. They are for the most part directed especially to the explanation of cases where nuclear and cell division have become definitely correlated, and in many cases they assume the determination of the plane of cell division by the axis of the nuclear spindle. Cases of cell division between resting nuclei, such as are found in the cleavage of the insect egg, have received less attention. Still, in all the sporanges studied it is plain beyond all else that nuclear division neither determines nor is in any way connected with cell division, and it is thus shown that the

cytoplasm can divide without any reference to the division of the nuclei. The cytoplasm both initiates and completes the process of dividing any particular cell mass, while the nuclei are throughout in the resting condition in Synchitrium; and, on the other hand, in Fuligo it can carry on the cleavage in essentially the same fashion simultaneously with nuclear division, without apparently being influenced in the least degree by the occurrence of the latter. In Fuligo the axes of the nuclear spindles and the planes of the cleavage furrows may be inclined at all imaginable angles to each other (*figs. 5–7, 8, 9*). It is always to be noted in these cases that the cleavage furrow in question is not the one destined to separate the daughter nuclei which are in process of formation at the time when the furrow is forming.

The cleavage always lags behind nuclear division, never separating any two daughter nuclei until they have reached at least the resting condition, or are themselves engaged in the next following nuclear division. This latter case, as found in Fuligo, is especially interesting as showing most clearly that the apparatus of nuclear and cell division is entirely distinct. The mechanism of nuclear division is in full operation while cleavage furrows are also forming in adjacent regions of the cytoplasm in entire independence of it. It is thus shown that no such conditions exist here as in endosperm formation in the lilies, where, after nuclear division is complete, a set of new connecting spindles are formed between the resting daughter cells. The mechanism of cell division in the endosperm is the same as in ordinary cell division, which follows at once upon nuclear division. It is presumptively the mechanism of nuclear division which is in operation in this simultaneous cell division, though operating independently of and subsequently to the completion of a long series of nuclear divisions. In endosperm formation we find no cases in which the nuclei are themselves dividing at the same time that cell plates are forming between them and adjacent nuclei which are also dividing. On the contrary, I have observed considerable evidence in the division of the pollen mother cells of the larch, in cases where the first nuclear division is not to be followed by

cell division, that the fibers of the connecting spindle are utilized, at least partially, in the formation of the spindles for the second nuclear division.

It is by no means to be argued from the fact that cell division and nuclear division are independent in these simple forms of plant life that the processes are not most intimately connected in the higher plants and animals, nor that the position of the nuclear spindle may not determine absolutely the plane of cleavage in these latter cases. In the higher plants there can be no doubt that the spindle, persisting after nuclear division, forms a cell plate and determines the plane of cleavage. On the other hand, the process of cell division in Fuligo shows very clearly that such a correlation is by no means fundamental or universal. Whether or not the nucleus in any fashion influences the orientation of the cleavage planes in this latter case, it does not do it by means of the karyokinetic spindle.

Wille (**25**) in a preliminary communication has reported the discovery of a type of division in multinucleated cells in which the nuclei participate in the formation of new cross walls. Just what the nature of the nuclear activity is in this case is not clear from the brief report referred to. It certainly represents a new and most interesting condition in multinucleated cells.

It is quite possible that the irregular cleavage of the fungus sporange indicates a primitive condition when nuclear and cell division are entirely independent processes, and that the correlation of the two has been gradually achieved in the evolution of the higher plants and animals. It is plain, therefore, that the theories of Heidenhain and Kostanecki (**5** and **11**) can have no application in the explanation of cleavage in these sporanges, and it is further plain that no theory which interprets cell division as a function of the karyokinetic figure can claim to be of fundamental value for the explanation of the process. Cell division may be much more definitely related to purposeful results in tissue formation when, as in the higher plants, it is accomplished by the mechanism of the spindle and cell plate. It is quite possible that the regular orientation of successive cleavage planes

so as to form tissues, *i. e.*, aggregates of cells with specific shapes and dimensions, only became possible after cell division came to be a function of the same mechanism which effects the division of the nucleus, but, as Strasburger (**22**) long ago pointed out, the process itself is by no means necessarily dependent upon the existence of this particular mechanism or of any correlation whatever with nuclear division.

Hofmeister (**8**) calls attention to the fact that the division of protoplasmic masses for the formation of reproductive cells is quite universally accompanied by loss of water and reduction of volume with increased density of the protoplasm. This phenomenon of contraction and loss of water is especially conspicuous in the process of spore formation in sporanges, as I have already noted. It seems a fairly natural assumption that the tensions set up in a mass of protoplasm which is contracting as a result of loss of water may be utilized in some fashion to produce the extremely irregular cleavage furrows which we observe in the early stages of spore formation. That these furrows, however, are not purely mechanical in their origin and analogous to the fissures that appear on the surface of a drying colloidal mass is shown, as I have noted elsewhere, by the fact that, although irregular, they never cut off segments containing no nuclei, and ultimately they produce approximately equal uninucleated spores. Some form of organization must be assumed to be present in the protoplasm which determines the progress of the cleavage so as to lead to a constant result. Still, even with this assumption, the possibility remains that the source of the energy which is thus controlled may be in the tensions produced by contraction due to loss of water.

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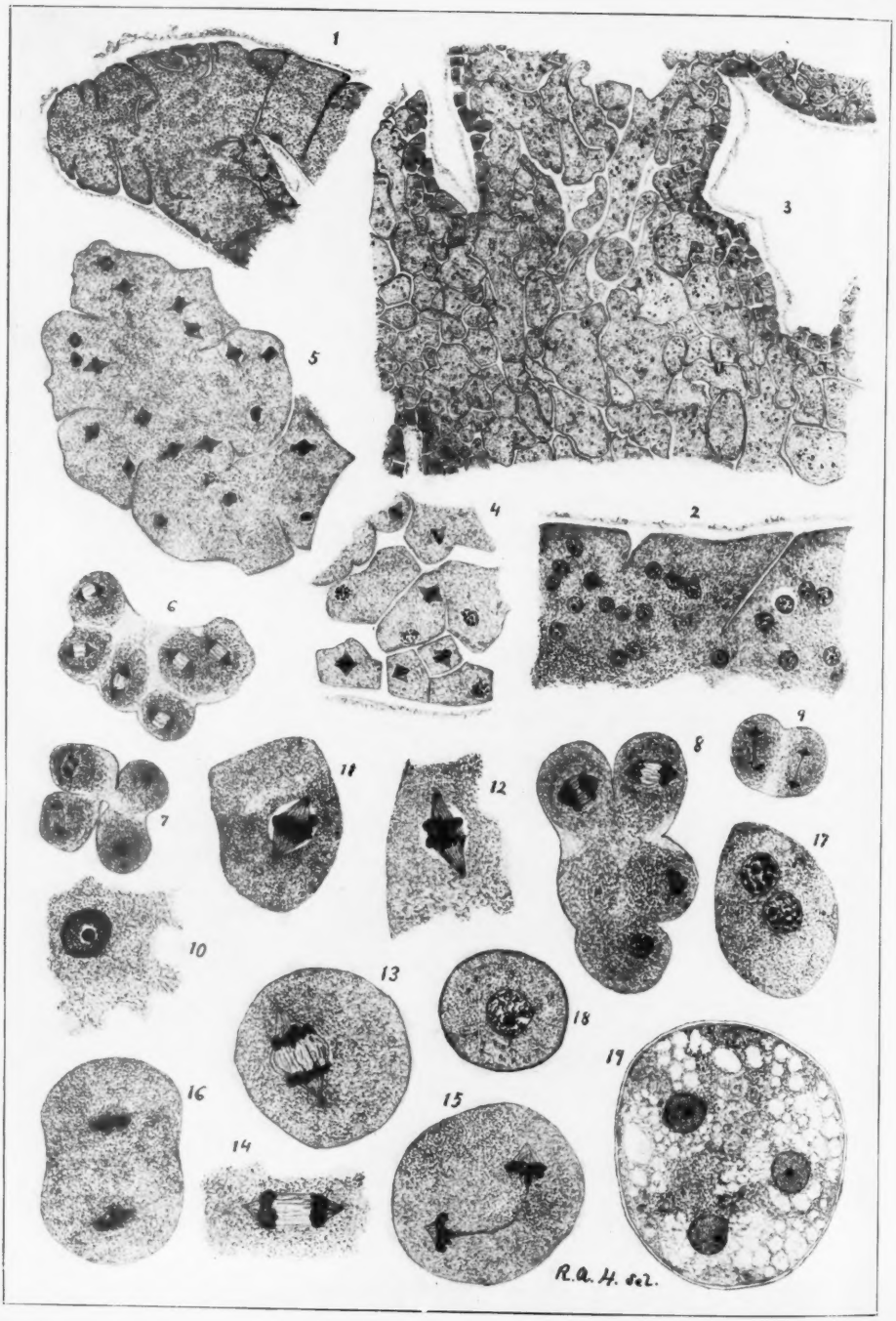

HARPER on FULIGO

EXPLANATION OF PLATE XIV.

All figures were drawn with the aid of the Abbé camera lucida, and all but *1* and *3* with the Zeiss apochr. obj. 2mm. *Figs. 2–7*, and *9* with oc. 6; *fig. 8* with oc. 12; and *figs. 10–18* with oc. 18.

FIG. 1. Portion of aethalium showing first cleavage furrows and lacunae of the protoplasmic reticulum. × about 150.

FIG. 2. Superficial portion of protoplasm showing two cleavage furrows and scattered nuclei.

FIG. 3. Portion of dividing protoplasm from interior of aethalium and lying between three lacunae marked *l*. × about 250.

FIG. 4. Several entire segments and portions of others from surface of lacuna in the interior of an aethalium; profile and polar views of nuclei in the equatorial plate stage.

FIG. 5. Segments from interior of aethalium with cleavage furrows cutting into it from various directions; nuclei in equatorial plate stage.

FIG. 6. Segment cut through by hyaline areas which predetermine the course of the cleavage furrows.

FIG. 7. Later stage in cleavage of a segment like that shown in *fig. 6*.

FIG. 8. Segment showing cleavage furrows cutting through undifferentiated protoplasm and others cutting into hyaline areas.

FIG. 9. Binucleated segment, the nuclei in a late stage of division and separated by a hyaline zone.

FIG. 10. Resting nucleus.

FIG. 11. Uninucleated segment; nucleus in equatorial plate stage; nucleole and part of nuclear membrane of parent nucleus still present.

FIG. 12. Metaphase; part of nuclear membrane of parent nucleus still present.

FIG. 13. Uninucleated segment; nucleus in later metaphase, connecting fibers curved.

FIG. 14. Later stage; connecting fibers straightened.

FIG. 15. Diaster; connecting fibers a narrow strand.

FIG. 16. Dispirem; cell division beginning.

FIG. 17. Binucleated segment; cell division not yet begun.

FIG. 18. Uninucleated spore with thin wall; granules of reserve material in cytoplasm.

FIG. 19. Encysted segment.

DOUBLE FERTILIZATION IN COMPOSITAE.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXI.

W. J. G. LAND.

(WITH PLATES XV AND XVI)

IN August 1898, Nawaschin communicated to the Russian Scientific Congress at Kieff the results of his work on fertilization in *Lilium Martagon* and *Fritillaria tenella*. Guignard, upon learning of the discoveries of Nawaschin, contributed to the Academie des Sciences a short account of his unpublished researches upon fertilization in several species of Lilium. Miss Sargant, from a reexamination of her preparations of *Lilium Martagon*, fully confirmed the observations of both Nawaschin and Guignard. A recent study of *Tulipa sylvestris* and *T. Celsiana* by Guignard gives results in strict accord with the earlier observations.

These observers find that both male cells upon emerging from the pollen tube are vermiform and twisted on their axes, suggesting the idea of non-ciliated spermatozoids. One male cell, coming in contact with the egg nucleus, retains for a time its vermiform shape, gradually enlarges until it becomes nearly spherical, and finally fuses with the egg nucleus. The other male cell fuses with the upper polar nucleus, and the nucleus resulting from this fusion unites with the lower polar nucleus. Sometimes the polar nuclei fuse and then unite with the male cell, and sometimes the polar nuclei and the male cell fuse simultaneously. Preparations of *Lilium Philadelphicum* and *L. tigrinum* in this laboratory show the last named condition. Miss Sargant figures one case in which the ends of the male cell are applied to the polar nuclei, uniting them as if by a bridge. Experiments in hybridization by De Vries and Correns seem to

indicate that double fertilization may be more frequent than is commonly supposed.

So far as recent work indicates, the spermatophytes produce two male cells. The persistent appearance of a second male cell, seemingly as well organized as the one which functions, has found no better explanation than a phylogenetic one, although it would be hard to explain why a cell which has long been abandoned continues to be well organized. We may well inquire whether a simultaneous fertilization of the egg and of the endo sperm nucleus may not be universal in angiosperms.

The study of the mature embryo-sac of Erigeron and of Silphium was undertaken for the purpose of determining the fate of the second male cell. The first named genus was chosen because a large number of ovules in different stages of development could be cut at once; the second, because of the differentiation shown by the disk and ray flowers.

MATERIAL AND METHODS.

Material was collected in the vicinity of Chicago, from June 25 to July 20, 1900. Collections were made at all hours of the day, and all material was killed in the field. The outer involucral scales of Erigeron were removed and the heads closely trimmed, only enough of the receptacle being left to hold the ovules together. A 1 per cent. aqueous solution of chromo-acetic acid was used as a killing and fixing agent. Carnoy's fluid was found to be unsatisfactory. The material was passed through xylol into paraffin and cut in serial sections 3.3 μ and 6.6 μ thick.

The method of treating Silphium was slightly different from that for Erigeron. The ovules, except those intended for tracing the path of the pollen tube, were freed from the surrounding tissues and immediately plunged into chromo-acetic acid at a temperature of about 100°, and were allowed to remain in the hot acid about two hours. After washing and dehydrating, they were passed through xylol into paraffin at a temperature of 63°. Sections 2–5 μ thick were cut on a Reichert rocking microtome.

Flemming's safranin-gentian-violet-orange G and Haidenhain's iron-alum-haematoxylin both gave excellent results, but the most satisfactory differentiation in fertilization stages was obtained with cyanin and erythrosin. This last combination, after the sections had been treated with acetic acid and chloroform, gave details of structure not obtained by other methods.

ERIGERON.

Two species, *E. Philadelphicus* L. and *E. strigosus* Muhl., were examined. The following statements apply to both, as there is no difference in their embryo sacs, except in size. The development of the embryo sac does not differ essentially from that of other Compositae. The number of antipodal cells varies, and the one nearest the endosperm nucleus is frequently binucleate.

The lower polar nucleus moves up and unites with the upper polar nucleus (*fig. 1*), which remains near the egg. The nucleoli are very conspicuous, with highly refractive bodies scattered through them.

The mature egg is pear shaped, its nucleus being very large (*fig. 2*) and showing a fine network of chromatin. A vacuole near the middle of the egg is very conspicuous.

The synergids are pear shaped, and the position of their nuclei is variable. The end nearest the egg invariably has a large vacuole, while the smaller end stains deeply and sometimes has a distinct constriction (*fig. 2*) between the nucleus and the tip.

The embryo sacs in the ovules upon the edge of the head are larger than those at the center, several cases being observed in which the sacs in the center of the head did not function. In *E. Philadelphicus* the usual length of the embryo sac when ready for fertilization is about 216 μ, the width being about 40 μ. The endosperm nucleus at this stage is from 14.4 to 19.8 μ in diameter, its nucleolus being usually 9 μ. The egg nucleus seldom varies from 10.8 μ, with a nucleolus measuring 5.4 μ. The endosperm nucleus is usually about 16 μ distant from the egg. Chamberlain has shown that in *Aster Novae-Angliae* the nucleolus

of the endosperm nucleus is very constant in size. Many measurements of the egg nucleolus of *E. Philadelphicus* were made, and in only one instance did it vary from 5.4 μ.

The male cells were not observed in the microspores, and no pollen tubes were found except in the embryo sac. When the pollen tube (*pt, fig. 3*) enters the sac, the synergids rapidly disintegrate, so that by the time fertilization is effected only fragments of their nuclei are visible. One male cell fuses with the egg nucleus, and at the time of fusion (*fig. 3*) cannot be distinguished from the egg nucleus. The other male cell fuses with the nucleus which is formed by the union of the polar nuclei, the product of this second fusion being the definitive or endosperm nucleus.

No male cells were observed in the pollen tube, or in the embryo sac before fertilization, so that nothing can be said concerning their previous appearance. The pollen tube, after it has discharged the male cells, usually contains two bodies of irregular shape (*fig. 3*), which stain intensely with cyanin. They were also observed in preparations of Lilium. These bodies may be mistaken easily for male cells, especially after fusion is complete. Nothing could be determined concerning their origin, although it is possible that they may have come from a division of the tube nucleus, since that still remains to be accounted for.

After a brief rest the definitive nucleus divides, and in the many preparations examined the cell plate was invariably parallel to the longer axis of the sac. The endosperm nuclei, after the last named division, are usually multi-nucleolate. The fertilized egg in the meantime shows little change, except a thickening of the wall and a slight enlargement; also it moves down nearer the center of the sac. At this stage scarcely any traces of the synergids can be seen.

In the second division of the endosperm nuclei the cell plate is usually at right angles to the long axis of the sac. The two upper nuclei resulting from this last division move towards the micropylar end of the sac, and, occupying the place made vacant by the synergids, lie a little above and close against the

egg. This last position may have been taken because of a movement of the nuclei in the direction of least resistance, which would be towards the place lately occupied by the synergids.

The fertilized egg usually completes its first division shortly after the second division of the endosperm, the first wall being transverse. The further development of the embryo does not differ from that of the other Compositae which have been investigated.

SILPHIUM.

Silphium integrifolium Michx., *S. terebinthinaceum* L., and *S. laciniatum* L. were studied. The last named species received more attention than the others on account of the large size of the ovules, and the ease with which they could be oriented for sectioning. Merrill's recent paper on the life history of the various species is so complete, except in regard to fertilization, that we will pass over all other stages.

As in Erigeron, the polar nuclei fuse long before fertilization, and the resulting nucleus comes to rest near the egg. A careful study of the microspores of *S. laciniatum* revealed the presence of long male cells, lying side by side and reaching almost around the interior of the spore. The pollen tube usually enters at one side of the synergids. In one preparation it was observed to turn almost at right angles, pass between the nucellar cap and the synergid, and then continue its course towards the egg. The synergid against which the pollen tube lies soon begins to show signs of disintegration, the other usually remaining intact until the fertilized egg begins to divide. In all preparations examined the pollen tube was much expanded above the nucellar cap, and its walls stained intensely.

Fig. 7 shows two small bodies (x) similar to those noted in Erigeron, which remain in the pollen tube after the male cells have been discharged. This figure also shows the coiled male cell (sp_1) resting against the egg nucleus (o). The second male cell (sp_2) is lying against the endosperm nucleus (e). The path by which it reached its destination can be seen

clearly. The cause of the peculiar appearance of the endosperm nucleolus shown in *fig. 7* is not known, but it is not believed to be due to reagents, as the other structures appear to be normal. *Fig. 8* also shows the coiled male cells lying against the egg and the endosperm nucleus. *Fig. 9* shows a more advanced stage of fertilization, the male cell fusing with the egg (*a*), having lost its coiled form. In the other (*b*), a trace of the coil may still be seen faintly. One is closely applied to the egg nucleus, the other to the endosperm nucleus. The irregular shape of the endosperm nucleus shown in *fig. 7* is also apparent here. Nawaschin, in a paper received since the above mentioned figures were drawn, describes coiled male cells in the embryo sac of *Helianthus annuus* and of *Rudbeckia speciosa*. His description of the reticulated porous structure shows them to be similar to those found in Silphium.

The large amount of food present in the egg is shown in *fig. 9*, the deeply stained bodies being starch grains.

After fusion with the male cell the endosperm nucleus divides rapidly (*ee*, *fig. 10*), and soon the sac is filled with a mass of nuclei. The fertilized egg (*fo*, *fig. 10*) does not divide immediately after fertilization, but rests for some time. It may be of interest to note that the nucleus of the upper antipodal becomes very large at this stage. The appearance of the antipodals suggests that they, for a time at least, may possibly serve to transmit food to the embryo, and also to the endosperm.

Merrill has shown that eight is the characteristic gametophyte number of chromosomes, and he believes that sixteen is the number in the tapetal cells. The writer was unable to make an accurate count, but sixteen appears to be the number in the embryo, and more than sixteen were counted in the endosperm. It is believed, as the result of many counts, that the number in the endosperm is twenty-four. This is the number we should expect here, for the primary endosperm nucleus results from the fusion of three nuclei, each containing eight chromosomes.

SUMMARY AND CONCLUSIONS.

In Erigeron the pollen tube passes down a short distance into the sac and discharges the male cells, which bore their way through the surrounding cytoplasm. One male cell fuses with the egg nucleus, the other with the endosperm nucleus. The endosperm nucleus, after fusion with the male cell, rapidly divides and soon fills the sac with a mass of nuclei. The fertilized egg remains some time in the resting condition, doing little beyond developing a dense membrane and becoming slightly larger. The first wall is transverse, as is usual in angiosperms.

In Silphium the polar nuclei fuse long before fertilization. The pollen tube passes down into the sac and discharges two male cells. These cells, in some instances, leave a well-marked path through the cytoplasm. They retain their coiled form for some time after contact with the nuclei with which they ultimately fuse. One fuses with the egg nucleus, the other with the endosperm nucleus. Before fusion is completed they become nearly spherical, being slightly flattened on one side.

I may venture to suggest that the fusion of the male cell with the endosperm nucleus is a true fertilization, and not a false one ("une sorte de pseudo-fecondation"), as regarded by Guignard. May not the two nuclei—egg nucleus and polar nucleus—nearest the middle of the embryo sac both be considered egg nuclei; and may not one of them, by some means not yet understood, have an advantage over the other, and so develop a more definite structure? May it not be possible that the fusion of three cells — the polar nuclei and the second male cell — to form the endosperm nucleus imparts to that nucleus an excessive stimulus to cell division, resulting in the rapid cell multiplication which immediately follows fusion?

That both the fertilized egg and the endosperm nucleus are potential sporophytes seems a reasonable view, yet we are confronted with the fact of the fusion of the polar nuclei, and until the meaning of this is made clear we must remain in doubt concerning the true significance of double fertilization.

The spermatozoid form of the male cell may be wide-spread in monocotyls and dicotyls. In all the forms recently examined they have been reported as present, either in the pollen tube, in the embryo sac, or lying against the nuclei. In Lilium and Tulipa the male cells seem to assume the vermiform shape in the pollen tube, since in the microspore they are spherical. In Silphium they assume the elongated form in the microspore.

At present double fertilization is known to be a fact in the Liliaceæ. There are indications that it will be found among the orchids. Among the dicotyls it has been found in *Delphinium elatum*, *Helianthus annuus*, and *Rudbeckia speciosa*. It has also been doubtfully suggested in Juglans. The present paper adds to the list *Erigeron Philadelphicus* and *Silphium laciniatum*.

The experiments of De Vries and Correns, and also of Webber, in hybridization of *Zea Mays*, indicate that we may confidently expect double fertilization to be reported soon in that species.

My acknowledgments are due Professor John M. Coulter and to Dr. Charles J. Chamberlain for assistance rendered during the progress of the work.

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EXPLANATION OF PLATES XV AND XVI.

All the drawings are made with the aid of a camera lucida, Bausch and Lomb $\frac{1}{12}$ obj. and ocular 1, giving a magnification of 1260. The plates are reduced to one half the original size. A Bausch and Lomb $\frac{1}{16}$ obj. was used for studying minute details.

PLATE XV. Erigeron Philadelphicus.

FIG. 1. Embryo sac with fusing polar nuclei, *e*; synergids, *sy*; egg nucleus, *o*.

FIG. 2. Mature embryo sac showing the long-beaked synergids, *sg*; egg, *o*; endosperm nucleus, *e*.

FIG. 3. Fertilization; pollen tube, *pt*, with two intensely stained bodies, *x*; male cell fusing with the egg, *a*; second male cell fusing with the endosperm nucleus, *b*.

FIG. 4. First division of endosperm.

FIG. 5. Later stage of endosperm division, showing cell plate and multinucleate nuclei.

FIG. 6. First division of fertilized egg.

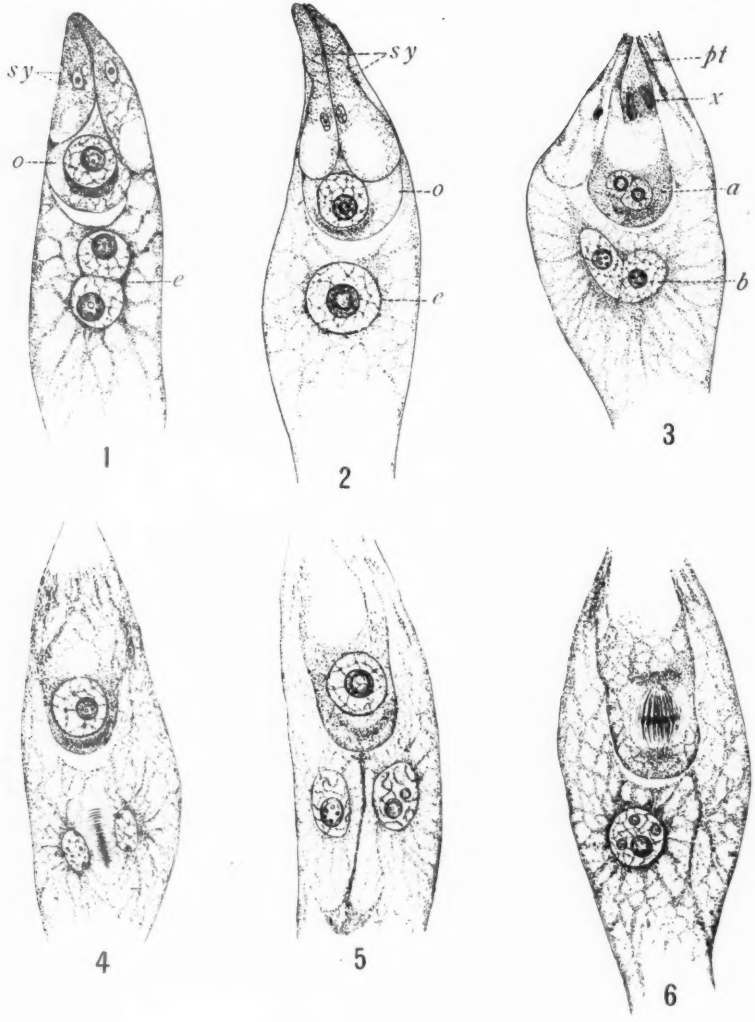
PLATE XVI. Silphium laciniatum.

FIG. 7. Fertilization; pollen tube, *pt*, passing into the sac; synergid, *sy*; unknown bodies, *x*; male cell, sp_1, lying against the nucleus of the egg, *o*; male cell, sp_2, lying against the endosperm nucleus, *e*.

FIG. 8. Synergid, *sy*; male cell, sp_1, lying against nucleus, *o* (a portion of this male cell was lost in sectioning); male cell, sp_2, with large coil, resting against endosperm, *e*.

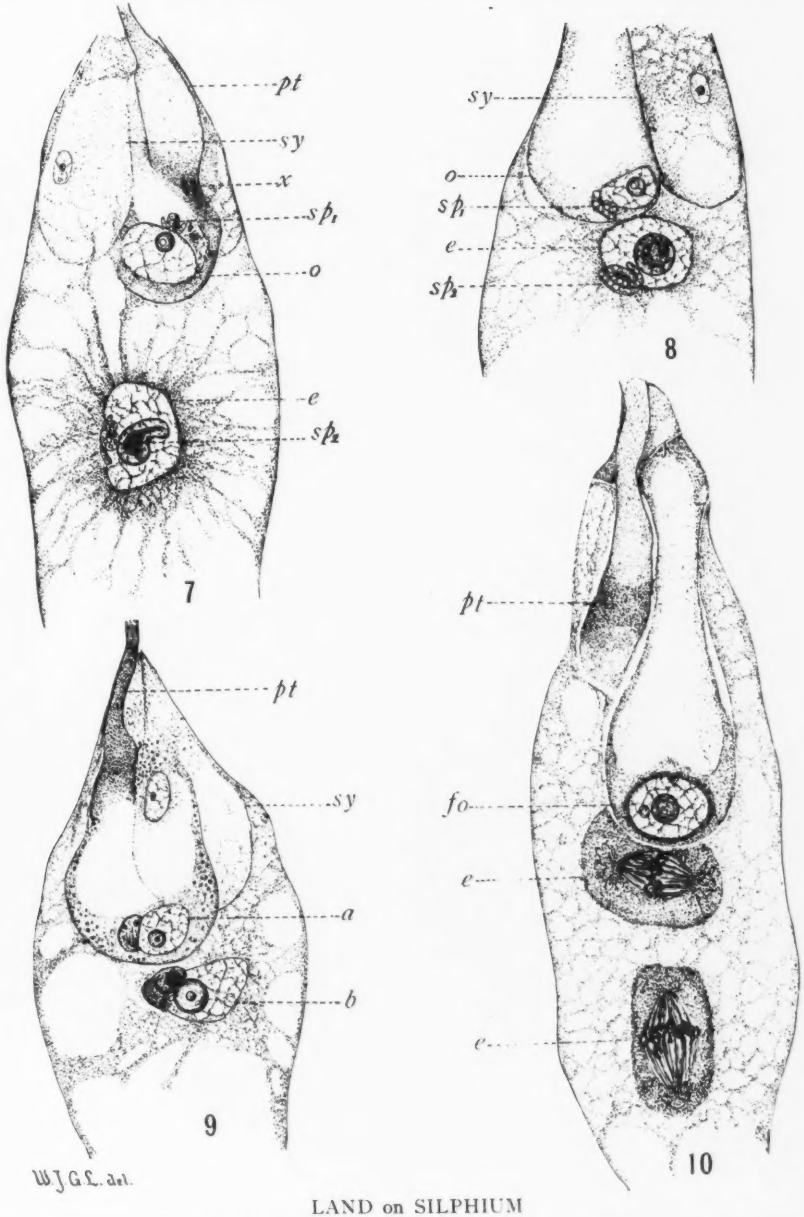
FIG. 9. Later stage of fertilization; pollen tube *pt*; synergid, *sy*; fusion of male cell with egg nucleus, *a*; fusion of male cell with endosperm nucleus, *b*.

FIG. 10. Second division of endosperm; pollen tube, *pt*; fertilized egg, *fo*; dividing endosperm nuclei, *e*, *e*.



W J G. L. del.

LAND on ERIGERON



LAND on SILPHIUM

A NEW CHROMOGENIC MICROCOCCUS.

MARY HEFFERAN.

(WITH FOUR FIGURES)

IN the course of an examination of river water for the Sanitary District of Chicago, carried on during the past year under the direction of Professor Jordan at the University of Chicago, a somewhat unusual opportunity has been afforded for the study of water bacteria. The water for this examination is taken from twenty-seven different stations along the Illinois and Desplaines rivers and their tributaries, and also from the Mississippi river above and below St. Louis. It is collected in sterile bottles with tightly fitting glass stoppers. As soon as the water is collected these bottles are enclosed in small tin cans which are packed with ice in larger cans, and shipped as speedily as possible to Chicago.

In making a qualitative examination of this water it has been plated in ordinary beef peptone agar (Witte's) and in Nährstoff Heyden agar.[1] The latter medium has the advantage of bringing to development a much greater number of colonies than ordinary peptone agar; for instance, at the end of ten days a peptone agar plate and the corresponding one of Nährstoff Heyden may present colonies in a ratio of 21 : 400. The reason for this enormous difference has not as yet been sufficiently determined. It is possible that the large, spreading, and rapidly growing colonies may inhibit the development of the more tardy forms on a peptone agar plate. On the Nährstoff Heyden plate these large colonies, particularly the fluorescent forms, are not seen, or at least appear only as small dots; while on the other hand, the plate is crowded with small, distinct, slowly developing colonies, some of which seem to present novel characteristics. It is likely, therefore, that this medium will furnish varieties not obtained on peptone media.

[1] Hesse and Niedner, Zeitschr. f. Hygiene 19 : 460.

Among the germs found in this way, I isolated several times during the course of the winter a micrococcus producing a salmon-pink pigment, which seemed not to have been previously described. This form first appeared in Mississippi river water, collected at "The Chain of Rocks," near the St. Louis water tower. The water in a dilution of 1 : 100^{cc} was plated in 15 acid gelatine and peptone agar, and in neutral Nährstoff Heyden agar, using 1^{cc} of the diluted water to each plate. The micrococcus developed only on the Nährstoff Heyden plate, as it did in other experiments with the same water, although on one occasion I found it upon an ordinary agar plate, showing that the special medium is not essential. It also develops readily upon agar in pure culture. Water from other stations along the rivers often gave rise to pink colonies on peptone agar or Nährstoff Heyden, which by casual observation could not be distinguished from those of the micrococcus. A closer examination, however, usually revealed in these cases a bacillus whose pigment upon cultivation showed less of a yellow tinge in the rose than that of the salmon-pink form. On one or two occasions these small pink colonies were found to be due to a yeast, but the bacillus was by far the more common form. The micrococcus may exist in other parts of the rivers, although I did not observe it in any other water than that of the Mississippi. My search for it was only incidental in connection with other work, and the notes upon its appearance refer it always to the locality mentioned.

In the two cases in which the germ was isolated and cultivated through all the stages, it occurred in close connection with a bacillus producing a yellow pigment. The first transfer from a rather crowded Nährstoff Heyden plate or from the more isolated colony upon the peptone agar plate resulted in a mixed culture of the two kinds, a second plating being necessary in order to separate them. The bacillus was not identified. The micrococcus, which it is the purpose of this paper to describe, grows well and is easily cultivated at room temperature in broth

and milk and on all ordinary solid media except potato. The cultural features are as follows :[2]

Gelatine. — On a gelatine plate at 15°–20° C. it grows slowly. The colonies were visible on the third day and appeared under low power as small irregular dots, transparent, but with a slight, thin, uniform granulation. On the next day those that had emerged upon the surface had lost their irregularity and had become perfectly circular and slightly larger; otherwise to the eye they were unchanged, no pigment being visible. Under a higher power the granulation appeared more distinct, the colonies seemed thicker and had a yellowish tinge in the center, and were thinner and more transparent at the edge. A few days later the surface colonies had taken on the characteristic salmon tinge. Under the low power they looked perfectly homogenous except for thinness at the periphery where the fine granulation showed more distinctly. The edges were smooth and clearly defined. Later only a very slow increase in size took place, the colonies reaching a few millimeters in diameter in several weeks.

Gelatine streak culture at 15°–20° C. showed growth on the third day in two delicate, finely wrinkled lines, one each side of the needle track. This was at first a pale creamy-pink in color. On the sixth day the lines were somewhat broader and had lost their delicate wrinkled appearance, the lower parts being confluent and smooth. Later the growth took on the true deep salmon color. There seemed to be almost no softening of the gelatine, except possibly a slight sinking of the line of growth.

Gelatine stab culture at room temperature showed a small cream colored colony on the surface and a slight development along the needle track at the end of the third day. The growth is very slow, the drawing (*fig. 1*) being taken from a three weeks old culture. The colony on the surface increases gradually

[2] Except where otherwise indicated the media used were prepared in accordance with the recommendations of the Bacteriological Committee. Jour. Amer. Pub. Health Assoc., January 1898.

in diameter and becomes more deeply colored, sometimes showing a slight zoning, the deeper pink in the center. The only evidence of liquefaction at first is that the surface of the gelatine sinks perhaps more rapidly than could be accounted for by ordinary evaporation, forming a hollow cup-shaped cavity lined by the smooth dry pink layer of the expanded surface colony. If the gelatine is rather stiff this surface sinking takes place very slowly. This dry cavity may reach two or three centimeters in depth in the course of a month (*fig. 2*). The deep growth along the needle track remains colorless and little developed.

FIG. 1.

FIG. 2.

Agar.—To the eye the agar plate colonies do not differ materially from those on gelatine. They appear on the fourth day at room temperature as minute dots, which take on a yellowish tinge. Examined under low power they are seen to be very irregular, and unlike the gelatine colonies they do not become smooth edged and round upon reaching the surface, but retain their irregularity, the edges showing a slight raggedness. The texture is uniform and very finely granulated, being somewhat more dense in the center and thinner on the periphery. They become salmon-pink in the course of a few days and increase very slowly in size (*fig. 3*).

Agar streak shows beginning of development in twenty-four hours as a slight whitish growth along the needle track. In forty-eight hours it has become a light pink, a much more creamy or yellowish-pink than the rose culture of *M. agilis* of a day or two older but of about the same development. Later the growth becomes quite luxuriant, gradually deepening in tint. It has much the same appearance as the agar growth of a pink yeast, Torula III, except perhaps for a more decided salmon color. On fresh, moist agar the edges are smooth and the whole growth a straight, shining,

FIG. 3.

moist layer. If the agar is older the edges are likely to be irregular or lobulated.

The reaction of the agar, ranging 15 acid, neutral, or 15 alkaline, Fuller's scale, produces little perceptible effect, although the early development may be slightly more rapid on neutral agar.

At 37° the growth is very limited, and there is no pigment, but growth proceeds normally on removal from the incubator. Light seemed to have little influence upon pigment production; cultures standing on a shelf near a north window where no sunlight entered had about the same appearance as those kept continually in a dark locker. Development on glycerine and 2 per cent. glucose agar showed no variations from ordinary growth.

Potato.—The micrococcus refused to develop on either acid or alkaline potato at room temperature or in the incubator.

Milk.—Cultures at 20° C. showed no change beyond a deposit and a thick surface ring of the same characteristic color as the agar and gelatine cultures. Lactose litmus milk brought out this color very well, the litmus itself turning a more vivid blue, indicating an alkaline reaction. In course of a month or more, the liquid showed traces of peptonization without coagulation.

Bouillon.—Became cloudy in forty-eight hours and remained so with no change beyond the slow accumulation of a salmon-pink deposit. No production of indol nor reduction of nitrate.

Fermentation tube.—Dextrose 2 per cent. showed slow growth in the bulb and accumulation of pink sediment. No cloudiness in the closed arm and no gas.

Staining reactions, morphology, etc.—The micrococcus stains readily with ordinary stains and is decolorized by Gram's method. Mounted preparations reveal small cocci measuring about 0.6 μ in diameter, grouped irregularly with no particular indication of sarcina, diplo- or strepto-coccus form. The unstained specimens in hanging drop showed no motility other than the Brownian vibration. They measure less than 1 μ, somewhat smaller than unstained specimens of *M. agilis*. No spores observed.

Upon cultivation in gelatine hanging drop under $\frac{1}{12}$ immersion lens, the cell multiplication appeared as shown in *fig. 4*. I was unable with this power to see the actual process of division, but there was little doubt that it took place in only two planes giving rise to a typical staphylococcus grouping. That division did not occur in three directions was evident from cultures in liquid gelatine or broth, in which the cells divided regularly into groups of two cells each, which remained together a short time, then separated into free single cocci. The best series observed was that figured in the drawing, the development occurring in a solid gelatine drop as follows: twenty-four hours after sowing, in which time multiplication is well begun, a group of three cells was brought into focus, carefully examined, and drawn at 11:30 A. M. I examined the group at intervals of a few minutes without seeing any signs of division, but at 2:30 it was evident that two cells had taken the place of one in the group. At 4:00 P. M. there were five cells, and two hours later (6:00 P. M.) an oblong group of six cells could be seen in one place. At 8:00 the next morning ten cells were clearly visible, and the group had begun to acquire the circular form characteristic of all the more advanced colonies in gelatine culture, although in agar hanging drop, as on an agar plate, the colony form remains irregular. At 2:30 P. M. of the second day of observation, only a slight increase had taken place, fourteen cells being in focus in one plane, forming a rosette-like figure. Afterward the growth was very slow, the last drawing of the colony having been made five days after sowing. All colonies showed the same glomerulus structure.

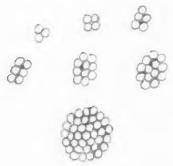


FIG. 4.

In the endeavor to identify this micrococcus with the pink varieties already described, I found some unexpected difficulties. First, the liquefaction of gelatine by this form is so slight as to pass almost without remark, making it doubtful in which of Migula's two classes the germ should be placed.[3] It is necessary, therefore, to examine his whole series, especially with

[3] MIGULA: System der Bakterien **2**: —. 1900.

regard to the two points most evidently characteristic of this form, its pigment coloration, and non-development on potato.

Migula describes eleven non-liquefying micrococci as *Rot wachsend*. Of these, seven may be thrown out, since they are distinctly said to have a seal, or carmine red, or vermilion pigment. Another is "gray on agar, red in milk, and does not grow at 37°." The other three are said to be "flesh red," the exact color of which is doubtful. Of these:

(1) *M. carneus* Zimmerman is described by Zimmerman himself[4] as having a deep flesh red, almost violet tinge, and growth on potato. Lustig describes a form, *Coccus ruber*,[5] found in water by Maschek which is probably identical with this.

(2) *M. sarcinoides* Migula (n. sp.)[6] looks often like sarcina, It is also described as a very large coccus, 1.8–2.5 μ in diameter. Nothing is said of the potato growth. Gelatine plate colonies reach 5^{mm} in diameter. Color on agar is a warm flesh red, more intense along the middle line. Bouillon has a flocculent whitish growth and a red sediment. This description, I think, hardly coincides with that of the salmon-pink form, although much depends upon what is meant by "flesh red."

(3) *M. rubellus* (Migula)[5] is morphologically scarcely distinguishable from the last, and shows the same sarcina grouping. The colonies are almost opaque, dark gray-brown. The description of the gelatine stick culture has some resemblance to that of the salmon-pink form, *i. e.*, a dry surface colony slowly spreading to walls of tube and having underneath a deep dry cavity of non-liquefied gelatine. But this growth is said to be a "bright flesh red." All cultures have a trimethylamine odor; on agar streak the edges are finely dentate, and bouillion has a red slimy sediment, enough points of difference to distinguish the forms.

In the class of germs which Migula describes as liquefying gelatine, we find out of the twelve named that three have carmine red pigment (*M. rubens*, *M. persicus* Kern, *M. haematodes* Babes); one is wine red (*M. rubiginosus* Kern); one has a violet

[4] Die Bakterien unserer Trink- und Nutzwässser. Chemnitz, 1890.

[5] Diagnostik der Bakterien des Wassers 40. 1893. [6] Op. cit.

tinge (*M. roseo-persicus*); and one (*M. fragilis* Dyar) is "brown-red on glycerine agar." These colors are not likely to be mistaken, and cut the list down to those which are either "rose" or "flesh red." Three of these (1) *M. roseus* Flügge, (2) *M. roseus* Eisenberg, and (3) *M. roseus* Maggiora are sometimes confused because of the similarity of name.

(1) *M. roseus* Flügge is only briefly mentioned by Flügge himself,[7] but is described more at length by Macé[8] as a form common in air, non-liquefying on a gelatine plate, slowly liquefying in a stab culture. But he says that the liquid portion is tinted red "more vermilion than by *prodigiosus*." Macé also mentions among species closely related to this, *M. agilis* Ali-Cohen, *M. rouge cerise* of List, isolated from water and producing a cerise color on potato, and Zimmerman's *M. carneus* which Macé, as well as Lustig, regards as identical with Maschek's *Coccus ruber*. He describes these as all non-motile cocci, about 0.8 μ in diameter, "rose red or flesh colored," and abundant on potato.

(2) *M. roseus* Eisenberg,[9] to which Migula gives the name of *M. subroseus*, is found in sputum and produces a pigment like that of *B. prodigiosus* on potato.

(3) *M. roseus* Maggiora is described by Ward[10] as a non-liquefying form 0.6 μ in diameter, associated in irregular glomeruli, and forming a pale rose pigment. I was unable to gain access to a further description of this micrococcus.

As to the remainder of Migula's list there is left:

(4) *M. cumulatus* Kern, a too definitely liquefying form to be identified with the salmon-pink micrococcus. Otherwise, excepting the fact that it is a facultative anaerobe, the descriptions are somewhat similar.

(5) *M. subcarneus* Migula shows in a gelatine stab culture after one day a small light red colony at point of incision. After three weeks a sirupy liquefaction begins in stocking-shaped

[7] Die Mikroorganismen 185 [3d ed].

[8] Traité practique de bactériologie 432. 1891.

[9] Bakteriologische Diagnostik 408. 1891.

[10] Annals of Botany **12**: 309. 1898.

form, with the colony on the surface and a thick, flocculent, rose-colored sediment. On agar, "rose" or "flesh colored."

(6) *M. rosaceus* (Frankland) is found in air. The entire description is as follows:[11] Cocci which are very variable in size, the largest 2.5 μ in diameter. On gelatine forms a smooth pink expansion on the surface, while needle track below remains almost undeveloped. In older cultures the margin assumes a radiated appearance, while still older cultures frequently exhibit slight liquefaction. On agar, smooth bright pink surface expansion, devoid of any further character. Broth after nine days is clear, free from pellicle, and has a pink deposit. On plate, the colonies appear to the naked eye as pin-head dots on surface, and are bright pink in color. Under low power ($\times$ 100) are seen to be of distinctly reddish tint, the edge irregular but smooth. As the colonies approach the surface, the irregularity diminishes.

Certain points in this description agree somewhat with that of the Mississippi river form, *i. e.*, the gelatine culture and plate colonies, but the size of the coccus, the agar and broth culture seem to differ, and the description is too imperfect to make the comparison at all complete. The same may be said of Frankland's description of his (7) *M. carnicolor*, the only stated points of difference from (6) being (*a*) a more rapid growth, (*b*) fainter color of pigment, and (*c*) slightly different appearance of colonies (more circular and brownish-pink). In both these forms the bouillon cultures are said to be clear after nine days, while numerous cultures of the salmon-pink form have remained cloudy for weeks.

This concludes the list of red growing cocci which I have found described, except that mention might be made of Bumn's *Diplococcus roseus* and *Sarcina rosea* (Schroeter), both liquefying air forms;[12] also *Sarcina mobilis* (Maurea),[13] which liquefies and will not develop on potato, but has a brick-red pigment. I have already mentioned *M. agilis* in the body of the description.

[11] Phil. Trans. Roy. Soc. London **178**: 269. 1887.

[12] EISENBERG, op. cit., 12 and 16.

[13] STERNBERG, Manual of Bacteriology 720. 1893.

It is evident that the color of the pigment produced by these germs, otherwise so similar, is of some importance in an effort to distinguish them. The descriptions are, for the most part, very meager in actual definite points, and it is doubtful if even the twenty-six standard tests given by Fuller[14] as showing a high degree of constancy would serve to differentiate these forms, so general in character are the indicated reactions of the table. In regard to the chromogenesis, it would be of no advantage to multiply the list of adjectives, for few bacteriologists could describe in this way the exact tints of "pink," "reddish" "rose," or "flesh color" with sufficient accuracy to make them unmistakable. An article by E. B. Shuttleworth,[15] on "Nomenclature of colors for bacteriologists" is one of the most recent attempts to systematize colors for bacteriology. This author's classification gives under yellowish-pink three subdivisions: salmon, salmon-buff, and flesh color. I was unable, however, to obtain the plates or illustrations to which his numbers evidently referred, and without them the words do not give a very distinct visual image.

A simple and more exact method of determining the pigment color of germs is that of the use of a color wheel. A little contrivance of the kind often used in morphology and easily obtained is a wooden top with the various small color disks. A better determination, because of the more constant rate of revolution of the disks, can be made with a Bradley color wheel and standard Maxwell disks. By this means I obtained the following result in the case of the salmon-pink germ.

An agar culture begins in a cream-pink growth for which the determination is not necessary. After about 5–7 days it begins to acquire the characteristic color, the composition of the color wheel for this age being near: red, 70 per cent.; white, 20 per cent.; yellow, 10 per cent.. Later the color deepens with the addition of a more orange tone, and remains almost constant after two weeks for all culture media—agar (different reactions),

[14] Jour. Expt. Med. **4**: 609. 1899.

[15] Jour. Amer. Pub. Herath. Assoc. **1895**: 406.

gelatine, on which the orange tint is slightly more marked, broth sediment, milk ring. Several trials to match this with the color wheel resulted in a range of:

	(1)	(2)	(3)
Red	76%	72%	72%
White	10	14	13
Yellow	8	9	9
Orange	6	5	6
	100	100	100

Of these, (3) was almost the exact tint of my cultures, with the one difference of a slightly duller tone than that shown by the actual growth, particularly of a rather bright gelatine culture. But any one of these combinations gives a good idea as to the characteristic tint.

By this method of description of colors there would be less possibility of confusion. The great advantage would lie in the systematization of those chromogenic germs whose color is fairly constant on ordinary media for certain ages. For instance, the whole *prodigiosus* group produces a color in liquefied gelatine which may be variously described in the vocabulary of different bacteriologists; but the fact that it closely approximates a color combination on the wheel of red 80 per cent., black 20 per cent., is sufficiently definite. *B. prodigiosus* and *B. ruber balticus* both give the above color to liquefied gelatine in a ten-day culture. In addition to this *B. ruber balticus* produces a heavy surface membrane of an entirely different shade, *i. e.*, red 80 per cent., orange 20 per cent.

The one difficulty for this accurate determination of pigment color is, of course, the liability of variation in the pigment produced by the same germ on media made by different bacteriologists or in cultivation under different circumstances. However, it has been found that if this organism is brought into good physical condition by a series of broth and gelatine cultures, according to Fuller's suggestion,[16] such variations in standard media are not of very wide range.

[16] Jour. Expt. Med. 4: 609. 1889.

Because of the peculiar salmon or yellowish-pink color of the pigment produced by the organism described in this paper, and because, although it is evidently closely allied to some of the pink micrococci previously described, it is not identical with any of them, I suggest for it the name **Micrococcus roseus flavus.**

In conclusion, I wish to express my sincere thanks to Professor Edwin O. Jordan for many valuable criticisms and suggestions during the progress of this work.

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BRIEFER ARTICLES

A NEW SPECIES OF NEOVOSSIA.

(WITH ONE FIGURE)

DURING September 1899, while collecting near Colo, Iowa, I found that the ovaries of *Phragmites communis* were affected by a peculiar smut. The specimen proved so interesting that Dr. Pammel, to whom I referred the matter, sent material to Dr. Farlow, who replied: "Your spores are perhaps a trifle smaller and have a slightly more marked epispore than in *Neovossia Moliniae*. Still the differences are very slight, and without further study I should not know whether to call them specifically different or not. You may have the material to determine this point." Accordingly Professor Hume, who is working upon the Ustilagineæ of Iowa, after a thorough examination and comparison of the specimens, decided that it was sufficiently distinct for a new species.

This genus was founded and distributed by von Thümen,[1] who called it Vossia. As this name had been given to a genus of grasses, Körnicke[2] changed it to Neovossia. Saccardo,[3] in his treatment of the Ustilagineæ, refers this fungus to *Tilletia Moliniae* (Thüm.) Wint., following Winter.[4] Dietel,[5] in describing the Ustilagineæ and Tilletiineæ, uses the Neovossia of Körnicke. Massee,[6] in his revision of the genus Tilletia, refers it to Neovossia, saying: "This species differs from Tilletia in the mode of spore germination, and must consequently return to Neovossia." Quite recently, Magnus[7] has discussed Neovossia, and considers it separated from Tilletia by the fact, discovered by Brefeld[8], that in germination the conidia do not copulate. He also considers that von Thümen has well founded the genus upon the fact

[1] Mycotheca universalis 1216. 1878; Oester. Bot. Zeitschr. **29**: 18. 1879.

[2] Oester. Bot. Zeitschr. **29**: 217. 1879.

[3] Sylloge Fung. **7**: 486. 1888.

[4] Rabenh. Krypt. Fl. Pilze **1**: 109. 1884.

[5] Ustil. u. Tillet. Nat. Pflanzfam. **1**: (Lief. 160). 1897.

[6] Bull. Roy. Gard. Kew 153, 154. 1899.

[7] Ber. d. deut. bot. Gesells. **18**: 73. 1900.

[8] Unters. Mykol. **12**: 210.

that the ripe spores, with the upper end of the sterigmata, break off from the full grown sterigmata. There seems to be sufficient reason, therefore, for establishing the genus Neovossia.

Neovossia Iowensis Hume and Hodson, n. sp.— Spore mass filling the ovary, black; spores globose, subglobose, or ovate, brownish-black, opaque, 16×20 to 24×28 μ, enclosed in a hyaline capsule; appendage slender, hyaline, two or three times the length of the spore; epispore apparently pitted.

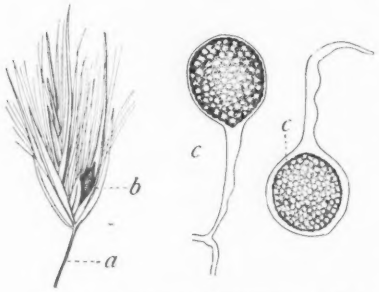

FIG. 1.—NEOVOSSIA IOWENSIS; *a*, spikelet of *Phragmites communis*; *b*, affected ovary; *c*, spores.

A careful comparison with specimen no. 1216 of von Thümen's *Mycotheca universalis* leads to the belief that the Iowa specimens are specifically distinct. The spores differ from those of *Neovossia Moliniae* (Thüm.) Körn. in being darker in color, broader and shorter, and generally blunter at the end opposite the appendage. The markings of the spore, also, are somewhat coarser. Ten spores of von Thümen's specimen, selected at random, gave an average of 27.7×17 μ, while the spores taken from the material collected at Colo, Iowa, gave 24.8×18.9 μ.— E. R. HODSON, *Ames, Iowa*.

NOTE ON THE ORIGIN OF TANNIN IN GALLS.[1]

THE origin of the different plant constituents is as much a mystery as their functions, and neither of these questions can be settled until more observations have been made. In considering the origin of tannin in galls the writer limits his observations for the present to the examinations of the common "ink-ball" or "ink-gall," which is produced on *Quercus coccinea* Wang., probably by *Cynips aciculata* O. S. The same kind of gall is produced on other oaks, as Mr. Beadle, of the Biltmore Herbarium, has sent me specimens which were produced on *Quercus imbricaria* Michx.

[1] Presented at the New York Meeting of the American Association for the Advancement of Science, June 1900.

I. These galls are produced during the summer months on the young branches and sometimes on acorns. When mature they fall from the trees and are nearly globular in shape, varying from 20–30^{mm} in diameter. They are solid throughout and of the consistency of the pulp of a green apple. Externally they are smooth, and in color are a mottled green, yellow, and brownish-red. At this stage they are made up of three distinct zones: (1) a central zone, made up of nearly isodiametric parenchyma cells packed with numerous small somewhat spherical or irregular starch grains which are colored blue with iodine; (2) the middle zone, composed of radially elongated parenchyma cells, possessing thick cellulose walls with prominent simple pores and containing a mass of protoplasm lying on the sides of the walls and a few starch grains (with the development of the egg of the insect there also appear in the cells of the middle zone numerous starch grains closely resembling those found in the central zone); (3) an external layer made up of irregular parenchyma cells somewhat collenchymatic in character, with the protoplasm as in the middle zone.

II. Decided changes take place soon after the galls fall from the tree: (1) A larva develops in the central zone and there are signs of activity in the protoplasm of the cells of this zone. A large nucleus with nucleolus lies centrally in the protoplasm and in some cases yellowish globular vesicles are apparent. These latter are fixed in the fresh specimens by means of copper acetate (7 per cent. solution), after which treatment they become more yellowish in color and insoluble in water, chloral, glycerin, potassium hydrate, or alcohol, and are no doubt in the nature of tannin vacuoles. (2) In the galls which contain a larva, and have been allowed to remain in copper acetate solution for several weeks or months, there separate in the parenchyma cells of the middle layer or zone yellowish crystals or crystalline masses, which are insoluble in water, chloral, glycerin, or alcohol. These crystals and crystalline bodies are lens-shaped, star-shaped, or fan-shaped, and much resemble the different carbohydrates, as hesperidin, inulin, etc., which separate in certain plant cells when the specimens are placed in alcohol. These crystals, however, do not separate in alcoholic material, and are to be found only in galls which have been kept in copper acetate solution. Their appearance, reactions, and a comparison with copper gallate crystals lead to the conclusion that they are identical in composition with the latter salt. (3) In the external layer or zone of specimens which are at this stage of maturity,

and have been treated with copper acetate, reddish-brown, amorphous, or somewhat crystalline masses are found adhering to the walls of the cells. These masses when amorphous are made up entirely of tannin and when somewhat crystalline contain an admixture of tannin and gallic acid.

III. When the winged insect has developed, (1) only a few layers of the cells of the central zone remain, and these contain a number of tannin vacuoles. Surrounding the latter are several (as many as 12) rows of prominent lignified cells. (2) The cells of the middle layer in specimens which are of this age and have been treated with copper acetate, contain numerous brownish-red tannin masses to which may be adhering some yellowish-brown crystals of gallic acid. But the tannin is in by far the greatest quantity in the cells of this layer and at this age of the galls. (3) The cells of the external layer also contain tannin masses.

Conclusion.—(*a*) It is well known that gallic acid occurs naturally in the nut galls (the product of *Cynips gallae tinctoriae* Olivier on *Quercus Lusitanica* Lamck.); the leaves of *Arctostaphylos Uva-ursi* (L.) Spreng., *Thea Chinensis* Sims, and of various species of *Rhus;* the fruit of *Terminalia chebula* Retzius (Myrobalans), and *Caesalpinia coriaria* Willd. (Divi-Divi); the acorn cups of *Quercus Ægilops* L. (Valonia); and may be obtained by extraction with water in the form of silky needles and asymmetric prisms. With the alkalies, alkaline earths, lead and copper salts, it forms crystalline compounds. (*b*) Tannic acid, on the other hand, is an amorphous substance and does not produce crystalline compounds with the salts mentioned. (*c*) Therefore, the crystalline compound found in the galls examined by the author is in all probability gallic acid. This appears to be formed at the expense of the starch during the chrysalis stage of the insect. With the maturing of the winged insect this is changed to tannic acid. The transformation of gallic acid into tannin appears to be one of simple condensation of two molecules of the former with the loss of one molecule of water, as follows: $2C_7H_6O_5$ (gallic acid) $= C_{14}H_{10}O_9$ (tannic acid) $+ H_2O$.—HENRY KRAEMER, *Philadelphia College of Pharmacy*.

CURRENT LITERATURE.

BOOK REVIEWS.

The Cyclopedia of American Horticulture.

THE second volume of Bailey's *Cyclopedia*[1] has just been issued, including the letters E–M. The general design and scope of this important work of reference has been set forth already in these pages.[2] To this nothing need be added beyond the statement that the second volume is even better than the first, a natural improvement as the editors attained greater familiarity with their work and materials.

Aside from the articles treating large and horticulturally important genera like Gladiolus, Iris, Mamillaria, etc., there are some notable articles on general subjects, which serve to illustrate well the wise plan of the book and the thorough treatment of topics. *Forcing* is discussed in its general aspects by Professor Bailey; forcing vegetables, by C. E. Hunn; forcing fruits, by William Turner; and forcing hardy plants, by B. M. Watson. *Ferns* are treated botanically by L. M. Underwood; growing hardy ferns, by Edward Gillett and F. W. Barclay; growing tender ferns, by N. N. Bruckner. Again, in the article *Grape*, the editor writes of the general and historical aspects, while specialists in the various grape-growing regions of the north, the south, and the Pacific slope write of the practical phases of grape culture, and another of grape growing under glass. In the same way the horticultural and historical features of the *Greenhouse* are treated by the editor; Lord and Burnham write of its structural details; glass for it is discussed by J. C. Blair; and the methods of heating by L. R. Taft.

Among the biographical articles, the one on *Asa Gray*, by the editor, is a model of what such writing should be, for here Professor Bailey writes *con amore*, and with clear insight and appreciation. Morphological articles are conspicuously wanting; but perhaps these ought not to be expected. It would seem, however, that fruits might have been discussed from this point of view, as *flower* has been. *Insects* are well treated, and in part morphologically by M. V. Slingerland.—C. R. B.

[1] BAILEY, L. H.: *Cyclopedia of American Horticulture*, comprising suggestions for cultivation of horticultural plants, descriptions of the species of fruits, vegetables, flowers, and ornamental plants sold in the United States and Canada, together with geographical and biographical sketches. Vol. II. E–M. 4to., pp. xiv + 512–1024, *figs. 744–1453, pls. 10–19*. New York: The Macmillan Company. 1900. $5.

[2] BOT. GAZ. **29**: 282. 1900.

Tobacco.

DER TABAK, by C. J. Koning,[3] treats of the commerce, manuring, culture, anatomy, diseases, and fermentation of tobacco. Well-known facts are here presented in a popular manner to those interested in the culture and trade of tobacco. Only the contents of the chapter on the so-called fermentation of tobacco are essentially new. This process the author ascribes to the action of bacteria, in accordance with the hypothesis of Suchsland. "The aroma of the tobacco is caused by facultative anaerobes in so far as we can speak of aroma in our Dutch tobacco." In this remark, from page 23, the bacteria are called "facultative anaerobes," while on page 53 we find the statement that the supposed principal generator of the aroma is an obligate aerobe. The reviewer is sorry to differ from the views of Koning, yet he has examined very carefully, with very high magnifying powers, wrapper leaves withdrawn directly from the interior of the fermenting heaps in Florida, without discovering any colonies or any coating of bacteria. The few isolated rods and cocci found on some square centimeters of leaf cannot possibly have any significance. The water content (18–25 per cent.) of fermenting wrapper leaves prevents not only the development of fungi, but still more, that of bacteria. Filler leaves are more heavily moistened, and may contain 35 per cent. of water, but even these do not show bacterial colonies when heated in closed vessels to 55° C., a temperature reached often by the heaps of fermenting tobacco leaves. Even 60° C. is often reached, and nevertheless the heaps will heat up again when taken apart and rebuilt afresh. This would be impossible if the *Bac. Tabaci* really generated the aroma, since this dies at 50° in thirty minutes, and at 60° in five minutes, according to Koning's own statement. At the same temperature also the *Diplococcus Tobaci Hollandicus*, which Koning claims improves the combustibility of tobacco, also succumbs.

In not a single instance has the author stated the water content of the tobacco when he started his bacteriological investigations. Tobacco with 50 to 60 per cent. water will no doubt readily develop bacteria, especially of the Proteus group which Koning found; cocci also will thrive in great numbers. Should this rotting be interrupted at the right time, a change of the odor might have occurred, which may be very desirable with Dutch tobacco. But such rotting is carefully avoided by the progressive American tobacco manufacturers, by keeping the water content so low as to avoid the action of bacteria. Under these conditions alone the oxydizing enzyms of the tobacco leaf will develop their activity, and to this the changes of odor and aroma characteristic of superior tobacco have to be ascribed.[4]—OSCAR LOEW.

[3] KONING, C. J.: Der Tabak, Studien über seine Kultur und Biologie. Small 4to, pp. 86. Amsterdam: J. H. & G. Van Heteren. Leipzig: Wilhelm Engelmann, 1900. *M* 4 (unbound).

[4] Compare Reports No. 59 and 65 of the U. S. Dept. of Agriculture.

MINOR NOTICES.

THE FIRST SUPPLEMENT to Paris's *Index Bryologicus* has been issued by the house of Georg & Cie, Geneva, as one of the *Mémoires de l'Herbier Boissier*. It contains 234 pages, with innumerable entries correcting and adding to the original *Index*.—C. R. B.

THE FOURTH PART of the new edition of Weisner's *Die Rohstoffe des Pflanzenreiches*[5] completes the account of plant fats (42 pp.); Dr. K. Mikosch treats vegetable wax (21 pp.); Dr. A. E. von Vogl writes the tenth section on camphor (6 pp.); Dr. S. Zeisel contributes the chapter on starch (80 pp.); and Dr. Lafar's section on yeast is begun (11 pp.)—C. R. B.

THE SECOND FASCICLE of the list of the genera of seed plants, according to the system of Engler, has appeared with remarkable promptness.[6] In the notice of the first part in this journal[7] the general character of the work was stated. In the present signatures 1220 genera are listed, bringing the number up to 2490, the list beginning with *Alophia* (Iridaceæ) and ending with *Silene* (Caryophyllaceæ).— J. M. C.

RECENT NUMBERS of Engler's *Die natürlichen Pflanzenfamilien* are as follows: Number 198 contains a continuation of *Musci*, by Carl Müller and W. Ruhland, and deals as yet with the general morphological characters of the group. Number 199 contains the conclusion of the Marattiaceæ by G. Bitter, the Ophioglossaceæ by the same author, and a general discussion of fossil Filicales by H. Potonié. Number 200 and 201 (a double number) contains the completion of the Hyphomycetes, by G. Lindau, and with it the completion of the part of the first volume which deals with the Fungi.— J. M. C.

DR. J. P. LOTSY gives a résumé in English[8] of several articles in German and Dutch, in which he has published the results of his investigations upon the localization and formation of alkaloids in Cinchona. The chief results may be stated briefly thus. Normally, alkaloid is present exclusively as the content of living parenchyma cells, or of other cells differing but little from parenchyma. It never occurs in cells containing oxalic acid (as oxalate). Usually it is dissolved in the cell sap in young organs, but is an amorphous solid in old parts. The alkaloid is formed in the leaves and transported to the bark, where it is stored either in its original form or, after transformation

[5] Pp. 481–640, *figs. 89–122*. Leipzig: Wilhelm Engelmann. *M 5*. See BOT. GAZ. **30**: 66. 1900.

[6] DALLA TORRE, C. G. DE, and HARMS, H.: Genera Siphonogamarum ad systema Englerianum conscripta. Fasiculus secundus (signatura 11–20). Small 4to, pp. 81–160. Leipzig: Wilhelm Engelmann. 1900. *M.* 4.

[7] BOT. GAZ. **30**: 67. 1900.

[8] Bulletin de l'Institut botanique de Buitenzorg. III. 8vo, pp. 43.

to another alkaloid. It is considered not at all impossible that the alkaloid is formed by direct synthesis, and not as a decomposition product of proteids.—C. R. B.

A SMALL PAMPHLET by W. Johannsen presents an account in popular form of a process which has already attained considerable use in forcing-houses.[9] The method, which extraordinarily hastens the development of shoots and flowers, consists in exposing dormant plants in a suitable chamber twice to ether vapor for 24–72 (mostly 48) hours, with 48 hours interval, the time depending on the temperature and phase of the resting period.

The first section, "Zur Orientirung über die Ruheperiod," has considerable theoretical interest. The author defines clearly the expression "resting period," and shows the erroneousness of the common idea that the riper the wood or seed of a plant is, the easier will be the budding or sprouting. He points out that the resting period has no sharp limits, but is a passage from diminished power of growth through complete rest to increased power of growth again.

In the second section detailed directions are given for the practice, which is especially applicable to syringas, azaleas, snowball, spireas, deutzia, lily of the valley, and tulips.—C. R. B.

THE FIELD COLUMBIAN MUSEUM has recently come into possession of a set of plants collected by Don José Blain on the Isle of Pines, Cuba, some time in the middle of the sixties. The list, including 185 numbers, among which are four new species (*Polygala*, *Salacia*, *Spigelia*, and *Heliotropium*), has appeared as one of the publications of the museum (1:425–439. 1900), under the title "Plantae Insulae Ananasensis," by Charles F. Millspaugh, the curator.—The same author has also issued a second paper under the title "Plantae Utowanae"(Field Columb. Mus. Bot. 2:113–135. 1900), in which he reconsiders the Cyperaceæ and Cakile of the former one (see BOT. GAZ. 29:360. 1900). The present paper takes up the two groups in the form to be used in the proposed *Yucatan Flora*, in which all of the specific descriptions are to be based upon the characters of the fruits, and accompanied by text cuts illustrating these characters. Dr. Millspaugh differs from C. B. Clarke in regarding Mariscus and Torulinium as but sections of Cyperus. *Cyperus* (Mariscus) *Caymanensis* is described as a new species. In *Cakile* ten species are recognized, two of which are new; also two new hybrids are described. The author makes the very interesting observation that the genus has laid special stress upon the development of the fruit for dissemination, and that the "evolution for floatage seems to have reached its height in the new species growing upon the Alacran shoals."—J. M. C.

[9] JOHANNSEN, W.: Das Aether-Verfahren beim Frühtreiben mit besonderer Berücksichtgiung der Fliedertreiberei. 8vo., pp. 28, *figs. 4*. Jena: Gustav Fischer. 1900. 80 pfg.

STIGMONOSE is the title of a bulletin by Albert F. Woods[10] in which he describes fully his studies of the disease of carnations and other pinks, formerly called bacteriosis by Arthur and Bolley, and ascribed by them to the action of *Bacterium dianthi*. The preliminary statement by Mr. Woods as to the cause of the disease and the tone of his criticisms on Arthur and Bolley's work[11] were criticised by this journal[12]. Mr. Woods has now presented the evidence on which his conclusions rest, and it entirely justifies the substance of his criticism. Moreover, the account of Arthur and Bolley's work in the bulletin is full, and the defects in it are pointed out in a way to which no exception can be taken.

Woods shows that neither fungi nor bacteria are present in the earlier stages of the disease, and though they may appear later, their presence is not constant. The punctures by aphides are responsible for the disease, as was shown repeatedly by colonizing aseptically these insects on carnations. As "bacteriosis" is misleading, stigmonose is suggested to replace it. Mr. Woods believes "that the insect injects some irritating substance of an acid or enzymic nature into the wound; that this substance causes the increase of oxidizing enzymes in the cells which it reaches, and that these enzymes interfere with the nutrition of the cell by destroying the chlorophyll and setting up other changes which finally result in death."—C. R. B.

A FRENCH TEXT on the anatomy and physiology of plants by Er. Belzung[13] shows that one can produce a very imposing and attractive looking book without contributing to the pedagogical advancement of a subject. The author is professor of natural sciences in the Lycée Charlemagne, and the author of various elementary text-books, two geological, one paleozoological, and one botanical, and one zoological, which have reached third and fourth editions. A corresponding work on the anatomy and physiology of animals has reached its eighth edition.

The author disclaims having written a treatise, but calls his book a work for the student in which he may obtain the fundamentals to prepare him for the study of completer works. But he will be an unfortunate student who bases his knowledge on such a book as this. Not that it is so full of errors, though it sins in this respect, but its correctness is purely mechanical. Moreover, erroneous impressions and ill-chosen points of view unfit it for its

[10] WOODS, A. F.: Stigmonose, a disease of carnations and other pinks. Bulletin 19, U. S. Dept. Agric., Div. of Veg. Phys. and Path. 8vo, pp. 30. *pl. 3*. Washington: Gov. Printing Office. 1900.

[11] A. A. A. S., Toronto meeting; see BOT. GAZ. **24**: 200–205. 1897.

[12] BOT. GAZ. **25**: 129–130. 1898.

[13] BELZUNG, ER.: Anatomie et physiologie végétales, al'usage des étudiants en sciences naturelles des universités, des élèves a l'institut agronomique, des écoles d'agriculture, etc. 8vo, pp. iv + 1320, *figs. 1699*. Paris: Felix Alcan. 1900.

announced purpose. For example, the "circulation" of the sap is repeatedly described and impressed by a diagram with arrows showing the direction of the "ascending sap" and the "descending sap." The "osmotic force" is presented as "une nouvelle force," residing in the protoplasm in virtue of which it exercises "une puissante attraction." Many other similar cases might be cited from all sections.

The best thing about the book is the illustrations, most of which are excellent. But as a whole it can hardly be commended.—C. R. B.

A NEW PART (second series, Part IV) of the *Minnesota Botanical Studies* has appeared, bearing the date August 15, 1900. It contains seven papers of varying length, and is altogether a worthy member of the series. "A contribution to the knowledge of the flora of southeastern Minnesota," by W. A. Wheeler, is in the nature of a report of the work of the State Botanical Survey during the summer of 1899, and the results are presented with a well-organized ecological background, accompanied by seven excellent plates from photographs showing characteristic vegetation features. "The seed and seedling of the western larkspur (*Delphinium occidentale*)," by Francis Ramaley, is a brief morphological and histological study, illustrated by a plate. "A preliminary list of Minnesota Erysipheæ," by E. M. Freeman, catalogues nineteen species, with their hosts. K. C. Davis publishes three important revisions which have been developed in connection with the work on Professor L. H. Bailey's *Cyclopedia of American Horticulture*. They are as follows: "Native and garden Delphiniums of North America," 52 species being described, one of which is new; "Native and cultivated Ranunculi of North America and segregated genera," the genera being *Batrachium* S. F. Gray (5 spp.), *Ranunculus* L. (96 spp., two new), *Kumlienia* Greene (1 sp.), *Ficaria* Huds. (1 sp.), *Cyrtorhyncha* Nutt. (1 sp.), *Arcteranthis* Greene (1 sp.), and *Oxygraphis* Bunge (1 sp.); "A synonymic conspectus of the native and garden Thalictrums of North America," 35 species being described. The final paper is entitled "Some preliminary observations on *Dictyophora ravenelii* Burt," by C. S. Scofield, accompanied by three excellent plates, and among the conclusions reached the following is of general interest: "There is in the young mycelial threads very good evidence of the occurrence of cell fusion previous to, or in intimate connection with, the formation of the sporophore." —J. M. C.

NOTES FOR STUDENTS.

NAWASCHIN[41] has recently made a cytological study of *Plasmodiophora brassicae* Woron. Plasmodiophora is a parasitic myxomycete which causes various deformities and distortions in the roots of its host. *P. brassicae* is

[41] Beobachtungen über den feineren Bau und Umwandlung von *Plasmodiophora Brassicae* Woron. im Laufe ihres intracellularen Lebens. Fora **86**: 404–427. *pl. 20.* 1899.

the cause of "club root" in cabbages and turnips. Woronin, who studied this form more than twenty years ago, found that it does not form sporangia, but that the spore masses lie free in the cells of the host. Eycleshymer, in *Jour. Mycol.* 7:79–88. 1892, gives a clear account of its life history and distribution in the United States. The present paper is concerned with the more minute details. Infected roots were cut into very small pieces and were treated with Flemming's stronger solution for twenty-four hours. The author thinks that the advantages of a more prolonged treatment are entirely imaginary. Sections were cut 2–3 μ in thickness, and were fastened to the slide with distilled water without any further fixative. The Flemming triple stain gave the best results. Dilute Delafield's haematoxylin, followed by eosin in clove oil, and also the gentian-violet method according to Gram, gave good results. The peculiar method of nuclear division in the vegetative amoebae is worthy of special mention. In the resting condition this nucleus has a membrane, a nucleolus, and an extremely delicate chromatin network. As division begins, clearly differentiated chromatin granules appear in the place of the network, the granules having no genetic connection with the nucleolus. A plate evidently derived from the chromatin granules is then seen near the nucleolus. At this stage there is a sort of one-sided "achromatic figure" with its base resting upon the chromatin plate and its apex at the nuclear membrane, but the figure afterward acquires a symmetrical aspect, the drawings in the plate bearing considerable resemblance to bipolar spindles. The nucleolus then divides transversely, and the two resulting bodies take positions on opposite sides of the chromatin plate, which is now a homogeneous disk with chromatin granules imbedded in its periphery. The chromatin plate then splits, and the two parts, each accompanied by a nucleolus, move toward the poles of the spindle, and two daughter nuclei are formed. This method of division is of greater interest because the nuclear division in the plasmodium is simultaneous and of the usual bipolar mitotic type. The author's summary of the entire paper is about as follows:

1. The group of infected cells arises by the repeated division of a primary infected cell.

2. During the growth of the infected cells numerous multinucleate amoebae appear, which multiply without fusing to form a plasmodium.

3. In this condition the amoebae of Plasmodiophora are remarkable for their structure, and especially for their unique mode of nuclear division.

4. The mode of nutrition of the amoebae seems to be different from that in other Myxomycetes.

5. As the amoebae fuse into a plasmodium, characteristic changes take place in the structure of the body and in the nuclei.

6. The formation of a plasmodium takes place only after the host cell is exhausted.

7. In the mature plasmodium spore formation is preceded by repeated nuclear division of the typical mitotic sort.

8. In the first period of its development the parasite does not kill the host cell, but merely causes it to hypertrophy.—CHARLES J. CHAMBERLAIN.

HERBERT J. WEBBER, who has long been investigating the subject of plant-breeding for the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture, has published [15] his results upon xenia in maize, which means the immediate effect of pollen upon structures outside of the embryo. His experiments are by no means complete, but they have already yielded suggestive results, and the author is justified in their publication by the fact that the subject has been brought under discussion by the discovery of double fertilization, and its application by de Vries and Correns to the explanation of xenia. Mr. Webber had already reached the same conclusion independently, and was collecting a large amount of experimental data to substantiate his more leisurely developing paper.

It seems that the name "xenia" was applied to this phenomenon by Focke.[16] While it is claimed to be a somewhat common phenomenon in many plants, there are very few cases on record that are not open to doubt, and in no plant is its occurrence so well substantiated as in maize. The experiments of Mr. Webber have been conducted since 1898, in Washington and at the Nebraska Agricultural Experiment Station. The greatest care was used to obtain pure races and to prevent the access of foreign pollen. Full details are given of about thirty experiments which yielded pertinent results, and the paper closes with their discussion.

The author abundantly confirmed Körnicke's statement that xenia is shown only in the endosperm. Color in the endosperm is frequently transmitted by the pollinating parent, but color in the pericarp is not. The chemical composition of the endosperm is also greatly affected by the pollinating parent, sweet corn crossed with dent or flint races producing smooth grains with starchy endosperm, and *vice versa*. All of the experiments favor the theory that xenia in maize is caused by the fertilization of the endosperm nucleus by one of the male cells. All of the grains showing xenia proved to be true hybrids. In the case of spotted grains the author proposes an interesting hypothesis by way of explanation. He suggests that the male nucleus may fail to fuse with the polar nuclei, and in such a case may be able to form a spindle and divide separately. In this event two races of free nuclei would be formed, and when the parietal placing and tissue formation begin, the two races might become intermixed. A second hypothesis explaining this phenomenon suggests that the male cell fuses with but one of the polar nuclei,

[15] Xenia, or the immediate effect of pollen, in maize. Bulletin 22, with four plates (one colored). Issued September 12, 1900.

[16] Die Pflanzen-Mischlinge 511. 1881.

the other polar nucleus dividing independently. This also would result in two races of nuclei which might become more or less intermixed before fixation in a tissue.

We await with expectation the minute investigation of the structures concerned, which should settle the question of double fertilization for maize.—J. M. C.

THE POWER of the infusoria to adjust themselves to certain changes in their nutrient medium is the subject of a paper by Atsushi Yasuda.[17] From a nutrient fluid in which they were growing normally, the infusoria were transferred to solutions of the following substances: saccharose, glucose, lactose, glycerin, KNO_3, $NaNO_3$, $MgSO_4$, KCl, NaCl, and NH_4Cl, in varying concentration. The organisms used for the experiments were *Euglena viridis*, *Chilomonas paramœcium*, *Mallomonas Plasslii*, *Colpidium colpoda*, and *Paramœcium candatum*. In general, excepting Euglena, these can withstand about 6 per cent. of glucose, while Euglena withstands 11 per cent. With the other sugars the ratio is nearly the same, but with glycerin it is about $\frac{1}{6}$ instead of $\frac{6}{11}$, as above. In the case of the electrolytes it is still greater, usually however less than unity. *Aspergillus niger* withstands over 50 per cent. of glucose and 21 per cent of $NaNO_3$ (Eschenhagen), and Zygnema adapts itself to 50 per cent. of saccharose and 20 per cent. of glycerin (Klebs). Thus we see that in this respect Euglena stands intermediate between the other organisms here studied and the lower algæ and fungi, studied by Eschenhagen, Klebs, and Richter. This may be of interest to those attempting to decide the question of the animal or plant nature of Euglena.

If we use De Vries' coefficients for comparing osmotic pressures, the solutions at the limit of adaptability are, in a very general way, nearly isosmotic. The fact that in the author's results there are many very pronounced exceptions to this statement is not to be wondered at, since he has not only subjected the organism to a strong solution of the substance to be tested, but has also deprived it of its proper food supply. This, it would appear, is a very weak point in all such experimentation as the present. All these results were obtained when the infusoria must have been in an unhealthy condition. In general, the limit to the adjusting power seems to be of an osmotic nature. When the organism is first placed in one of these strong solutions its membrane becomes wrinkled, owing, doubtless, to extraction of water. Later these folds disappear; this is apparently the effect of an osmotic change in the protoplasm, adapting itself to the new conditions. The more concentrated the solution becomes, the more rounded is the form of the organism, and its outline becomes uneven.—BURTON EDWARD LIVINGSTON.

[17] Studien über die Anpassungsfähigkeit einiger Infusorien an concentrirte Lösungen. Jour. Coll. Sci. Imp. Univ. Tōkyō **13**: 101–140. *pls. 10–12.* 1900.

DR. TH. BOKORNY discusses[18] the various modes of storage of proteids and their microchemical relations. Proteids soluble in 5–10 per cent. salt solution (globulin) are stored in the proteid grains and crystals ("aleurone" and "crystalloids") of seeds. Proteids insoluble in NaCl solution have not been observed in proteid grains. Neither "active proteid" nor fat could be detected in the proteid grain itself; the fat is associated with the general proteids of the seed, probably with the plasmatic proteids. The plant caseins seem not to occur in the proteid grains, for these dissolve completely in NaCl solution, whereas the caseins are not soluble therein. The glutens of the cereals constitute a special case; they dissolve in 70–80 per cent. alcohol, a fluid which usually serves to precipitate proteids. Peptone was not recognizable in resting seeds. It and peptonizing enzymes occur in plants only exceptionally, as in fungi and carnivorous plants. Simple amides (asparagin, tyrosin, leucin, etc.) are well known in seeds and are widely distributed in vegetative parts. They appear to be the first decomposition product as well as the first formative stage of the proteids.—C. R. B.

L. KNY has been unable to find any traces of the intercellular living protoplasm said by Baranetzki to be observable in the intercellular spaces of young stems of *Myriophyllum spicatum* and *Ceratophyllum demersum*, and in young petioles of *Nuphar luteum*. Sauvageau also claimed to have found intercellular protoplasm in roots of *Naias major*. Kny examined a number of water plants and says: "In no case was I successful in observing living protoplasm (whether with or without nuclei or chromatophores) as a lining in the young or full grown air spaces except when its origin from the surrounding cells was in the highest degree probable. Even in the most advantageous covered preparations, in which the protoplasm within the cells adjoining the air spaces proved motile for several (in extreme cases fourteen) days, no sign of self-movement in the periphery of the air spaces was to be seen. The existence of a living extracellular plasma in the large air spaces of water plants must remain improbable until proof more convincing than at present is forthcoming."—C. R. B.

HERMANN FISCHER concludes his paper on "Der Pericykel in den freien Stengelorganen"[19] as follows:

1. In about 32 per cent. of the dicotyledons investigated a more or less perfect endodermis may be recognized marking the distinction between cortex and central cylinder. The so-called pericycle, *by its position* between the limit of the cortex and the ring of vascular bundles is allied with the pericambium of the root. Considered histologically, genetically, and as a formative region, no characters common to pericycle and pericambium can be predicated.

[18] Bot. Cent. **82**: 289–306. 1900. [19] Jahrb. f. wiss. Bot. **35**: 1–27. 1900.

2. In monocotyledons, conifers, and 68 per cent. of the observed dicotyledons no characteristic limit of the cortex is recognizable. The ring of mechanical tissue in the monocotyledons is from no point of view allied to the pericambium.—C. R. B.

DR. F. CZAPEK describes[20] a thermostat for use in experiments involving the use of a clinostat. The apparatus consists of a rectangular iron sand bath on four legs with leveling screws. This is heated by a microburner and over it is set a copper box sheathed with asbestos and equipped with the usual thermoregulator and thermometer. The ends of the box are slit to pass over the axis of the clinostat which carries the experimental plants. These slits can be closed by slides, except a circular aperture, which can be centered about the axis of the clinostat by means of the leveling screws of the base. Temperatures may be maintained constant within a degree for several days. The apparatus may easily be modified to furnish one-sided or uniform illumination by making part or all of the box of glass. A saturated atmosphere can be obtained by using water instead of sand in the pan.—C. R. B.

CHARLES PALMER NOTT has published an account[21] of the species of *Nitophyllum* of California. The paper is more than a description of the species, for it contains a general discussion of the generic characters and of the geographical distribution. It seems that this genus of the red algæ has a general distribution throughout the oceans of the globe. About seventy species are known, ten of which occur on the west coast of North America, and eight of these are limited to California or neighboring shores. *N. corallinarum* is described as a new species. The plates are photolithographs, and show the plant forms and even the texture excellently.—J. M. C.

[20] Ber. d. deutsch. bot. Gesells. **18**: 131. 1900.

[21] Proc. Calif. Acad. Sci. III. **2**: 1–46. *pls. 1–9*. 1900.

NEWS.

DR. B. M. DUGGAR of Cornell University has been elected a member of the German Botanical Society.

DR. H. AMBRONN, private docent in the University of Leipzig, has been called to the assistant professorship of botany in the University of Jena.

THE DIVISION of Vegetable Physiology and Pathology has secured a table at the Marine Biological Laboratory at Woods Hole for the use of its staff during the summer months.

BOTANY, a text-book for schools by Professor L. H. Bailey, is announced by the Macmillan Company. It is stated that "this book has all the popular features of the old time text-book — organography, morphology, elementary anatomy, and a flora — but it presents them in a new way."

DR. HERMANN VON SCHRENK is continuing his work for the Division of Vegetable Physiology and Pathology on the diseases of forest trees. As a result of the work of last year two bulletins have been prepared and are now going through the press. Dr. von Schrenk is at present on the Pacific coast, where he will remain for several months collecting information and arranging for more extensive work later.

MR. M. A. CARLETON, of the Division of Vegetable Physiology and Pathology, has completed the installation of the cereal exhibit of the Department of Agriculture at the Paris Exposition, and is now in southern Russia collecting grains, which will be distributed by the Section of Seed and Plant Introduction. A number of the varieties of cereals obtained by Mr. Carleton during his former trip to Russia have proved of value.

EARTH AND AIR is the title of a new monthly journal devoted to meteorology. The general purpose of the journal, as indicated by the prospectus and by the contents of the first number, is so pertinent to the work of ecologists and students of plant diseases, that the attention of botanists should be called to it. In the first number, Mr. Albert F. Woods, of the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture, writes concerning "Weather and plant diseases." The yearly subscription price is one dollar, and the office of publication is Penn Yan, N. Y.